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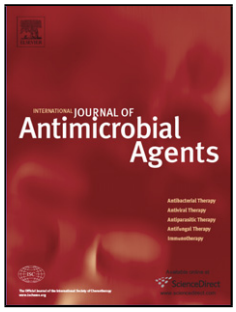

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Antibacterial properties of compounds isolated from *Carpobrotus edulis*

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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. Among 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]. Among 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 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^{TM} (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 reach 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^{TM} 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 an 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. Oleanolic acid had the greatest activity against the reference *M. tuberculosis* H37Rv strain.

3.2. Modulation of resistance in bacteria

One of the approaches to find new therapies against multidrug resistance is to search for compounds that increase the susceptibility of the organism to the antibiotics to which it is resistant. Use of such compounds as adjuvants results in a form of synergism that renders the inactive antibiotic active [38].

The methanolic extract of *C. edulis* was previously shown to have no in vitro antibacterial activity against *S. aureus* or *M. tuberculosis* strains. However, because it enhanced the killing of these bacteria post phagocytosis [6,9], regardless of the presence or absence of in vitro activity, all the compounds were evaluated for their ability to reduce or the reverse resistance of pathogenic bacteria to antibiotics to which they were resistant as well as for their activity on the efflux pump system of these bacteria by the semi-automated EtBr method.

Assays evaluating the modulation of antibiotic resistance by a non-antibiotic are performed as follows. First, the MIC of each antibiotic to which the bacterium is resistant and the compound that is to be assayed for modulation of resistance is determined. Second, the assay is repeated for each antibiotic at concentrations from its MIC to one that is deemed as ‘clinical susceptibility’ in the absence and presence of a concentration of the non-antibiotic that has no effect on the growth of the bacterium. The concentrations chosen for each compound were ≤0.5× MIC. The results of this modulation assay are presented in Tables 2–5. A minimum four-fold reduction in the MIC of an antibiotic by a given compound was considered significant.

3.2.1. Modulation of antibiotic resistance of Gram-negative strains

The ability of oleanolic acid, uvaol and epicatechin to decrease resistance of *E. coli* $AG100_{TET8}$ strain to tetracycline is shown in Table 2. These compounds decreased the MIC of tetracycline from 25 mg/L to 6.25 mg/L. However, their activities were not equal inasmuch as the reduction in MIC by some of these compounds required higher concentrations (e.g. the effective concentration of epicatechin was 100 mg/L compared with that of oleanolic acid which was 50 mg/L). With respect to oleanolic acid, a concentration of 50 mg/L apparently had reached a saturation of the target (efflux pump) such that higher concentrations of this compound would not increase its effectiveness.

Evaluation of the isolated compounds for reduction in the MIC of ciprofloxacin against *Salmonella* strains resistant to ciprofloxacin demonstrated that significant reductions could be achieved only by uvaol, MGDG and epicatechin and only for the strain *S*. Enteritidis 5408_{CIP} (Table 3). It is important to note that *S*. Enteritidis 104_{CIP} strain that has been induced to high-level resistance to ciprofloxacin is not affected by any of the compounds. Because resistance to ciprofloxacin in this strain is the result of an increased AcrB transporter, mutations in gyrase 1A and two mutations in the stress gene *soxS* [31], the inability of the compounds to reduce resistance of *S*. Enteritidis 104_{CIP} to ciprofloxacin suggests that the mutated targets beyond the efflux pump itself, such as gyrase, are not sensitive to the isolated compounds.

3.2.2. Modulation of antibiotic resistance of Gram-positive strains

An MRSA clinical strain was used to test the ability of *C. edulis* compounds to reduce the MIC of oxacillin, an antibiotic to which that strain is resistant. Resistance of

MRSA to β-lactams is due to the acquisition of *mecA*, a genetic element that carries the resistance gene for this class of antibiotics [39–41]. The origin of this genetic element remains unknown. The *mecA* element is known to be lost during exposure to an antibiotic of a different class and hence the organism becomes susceptible to β-lactams [42]. MRSA COL strain whose resistance to oxacillin had been increased from 400 mg/L to 1600 mg/L (i.e. MRSA COL_{OXA}) [28], was challenged with each of the compounds for the purpose of determining whether any of the compounds would be able to reduce resistance of this strain to oxacillin. The results presented in Table 4 demonstrate that only uvaol reduces the MIC of oxacillin. None of the compounds, as noted by Table 5, reduced the MIC of oxacillin against the MRSA clinical strain.

3.3. Activity on accumulation of ethidium bromide

3.3.1. Gram-negative bacteria

The semi-automated EtBr method affords real-time detection of EtBr accumulation inside cells by following the evolution of EtBr fluorescence over a period of time. It can be used to evaluate a compound for modulation of accumulation/efflux of EtBr. The modulating activity of a compound increases accumulation and decreases efflux of EtBr, presumably by having an effect on the activity of an efflux pump system. Because of the multiplicity of efflux pumps in Gram-negative bacteria, one cannot at this time specify any given efflux pump as being the one that is affected. However, the main efflux pump of Gram-negative bacteria such as *E. coli* and *Salmonella* is the AcrAB pump [30,43]. The semi-automated EtBr method has been used to screen for activity of purified compounds against the AcrAB efflux pump of *E. coli* strains that have been genetically characterised for the degree of expression of genes that

regulate and code for the AcrB transporter [30,35,43]. The effect of each compound on the accumulation and efflux of EtBr by *E. coli* strains that have been characterised for their efflux pump activity [30,35,43] was conducted using the semi-automated EtBr method. If compounds are to serve an eventual clinical role for activity against efflux pumps of Gram-negative bacteria that colonise the colon, such as *E. coli*, the pH of the medium should be close to that of the colon where the infecting organism resides, namely a pH close to 7. As evident from Figs 1 and 2, the compounds catechin and epicatechin were the most effective compounds for increasing the accumulation of EtBr in strain *E. coli* AG100 and hence were assumed to inhibit the intrinsic efflux pump system of *E. coli* AG100. However, the effects of epicatechin and catechin on the accumulation of EtBr are inhibited by the presence of glucose. These two compounds do not affect the accumulation of EtBr by the multidrug-resistant (MDR) *E. coli* $AG100_{TET8}$ strain at the concentrations used. The compound oleanolic acid had a modest effect on the accumulation of EtBr (Fig. 3) and this effect was also inhibited by glucose.

3.3.2. Gram-positive bacteria

Because it was observed that some of the compounds reduced the MIC of oxacillin against MRSA COL strain [28], the EtBr accumulation assay was employed to see whether these compounds could affect accumulation/efflux of EtBr by that strain. The MRSA strain used was the MRSA COL adapted to 1600 mg/L of oxacillin (i.e. MRSA COL_{OXA}) since it was this strain for which the MIC of oxacillin was reduced by some of the isolated compounds. Furthermore, use of the EtBr assay would also provide an understanding of the physiological conditions that could modulate any noted effect by the compounds on accumulation/efflux of EtBr. As shown by Fig. 4, uvaol increased

the accumulation of EtBr by the MRSA COL_{OXA} in a glucose-dependent manner. Similar results were obtained with MGDG (data not shown).

4. Discussion

Screening for in vitro activity of the compounds isolated from *C. edulis* against Gram-negative and Gram-positive bacteria and mycobacteria indicated that none of the compounds were active against Gram-negative bacteria and that some of them were very active against certain Gram-positive bacteria and had moderate activity against *M. tuberculosis*.

Activities against the MRSA COL_{OXA} and *S. aureus* HPV 107 strains are of significant clinical importance. In particular, oleanolic acid can be considered to exhibit high activity against the *E. faecalis* strain inasmuch as its MIC of 6.25 mg/L compares favourably with the susceptibility of this strain to common antibiotics [32]. The activity of oleanolic acid against the *M. tuberculosis* H37Rv strain is in agreement with the results of others [22].

Previous results showed that the methanolic extract of *C. edulis* could reduce or reverse the resistance of specific bacteria to antibiotics [6]. None of the compounds reduced the resistance of *S*. Enteritidis 104_{CIP} and MRSA to antibiotics to which they were resistant. This suggests that these compounds did not affect the efflux system of these organisms. However, some of the compounds reduced or reversed resistance to some antibiotics in other strains. Among these compounds, uvaol was the compound with the greatest ability to reduce resistance of the MRSA COL_{OXA} strain to oxacillin, the antibiotic to which it was initially resistant. The MRSA COL_{OXA}

strain owes its MDR phenotype to an overexpressed efflux pump system [28]. The inhibitory effect of uvaol on the accumulation and efflux of EtBr by the MRSA COL_{OXA} strain is glucose-dependent, suggesting that the effect of uvaol on the reduction of oxacillin resistance is due to its effect on the efflux pump system of this organism. Reversal of resistance of other MDR strains to given antibiotics produced by uvaol is also attributed to its effect on their efflux pump system.

Although many compounds have been shown to affect the activity of efflux pumps [44], none have yet been successfully used in a clinical setting as adjuncts with conventional antibiotics for the therapy of MDR bacterial infections. Moreover, most of the compounds that have efflux pump inhibitory activity are also toxic [45,46]. Because uvaol is apparently non-toxic [47] at concentrations employed in this study, it promises to be a good candidate as an adjunct to conventional therapy of MDR bacterial infections mediated by an overexpressed efflux pump.

Oleanolic acid, a compound with a similar skeleton to uvaol, was, together with catechin and epicatechin, moderately active in modulating accumulation and efflux of EtBr by *E. coli* strains. Oleanolic acid is known as an inhibitor of protein kinase, whilst flavonoids have been shown to inhibit the transport of glucose. These two mechanisms prevent the derivation of energy required for efflux pump activity, therefore accumulation takes place. In the presence of glucose, the capacity for inhibition of the efflux pump by oleanolic acid, catechin and epicatechin is lessened, as expected.

The effects of epicatechin and catechin on the accumulation of EtBr by *E. coli* AG100 are inhibited by the presence of glucose. This inhibitory effect was repeatable as noted by four different experiments. The reason for this glucose-dependent inhibition of the effects of epicatechin and catechin on the efflux pump system of *E. coli* AG100 remains unknown although it is under current investigation.

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Competing interests

None declared.

Ethical approval

Not required.

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Fig. 1. Effect of catechin on the accumulation of ethidium bromide by *Escherichia coli* AG100 in the presence and absence of glucose (0.4%).

Fig. 2. Effect of epicatechin on the accumulation of ethidium bromide by *Escherichia coli* AG100 in the presence and absence of glucose (0.4%).

Fig. 3. Effect of oleanolic acid on the accumulation of ethidium bromide by *Escherichia coli* $AG100_{TET8}$ in the presence and absence of glucose (0.4%). *Escherichia coli* $AG100_{TET8}$ refers to *E. coli* AG100 strain exposed to increasing concentrations of tetracycline leading to an efflux pump-overexpressing strain.

Fig. 4. Effect of uvaol on the accumulation of ethidium bromide by meticillin-resistant *Staphylococcus aureus* (MRSA) COL_{OXA} in the presence and absence of glucose (0.4%). MRSA COL_{OXA} refers to MRSA COL adapted to 1600 mg/L oxacillin.

Table 1

Minimum inhibitory concentrations (MICs) of purified compounds from *Carpobrotus edulis* on Gram-negative and Gram-positive bacteria and mycobacterial strains

Strain [a]	MIC (mg/L) [b]					
	β-Amyrin	Oleanolic acid	Uvaol	MGDG	Catechin	Epicatechin
Escherichia coli AG100	>200	>200	>200	>200	>200	>200
E. coli AG100$_{TET8}$	>200	>200	>200	>200	>200	>200
Salmonella enterica serotype Enteritidis 104	>200	>200	>200	>200	>200	>200
S. Enteritidis 104$_{CIP}$	>200	>200	>200	>200	>200	>200
S. Enteritidis 5408	>200	>200	>200	>200	>200	>200
S. Enteritidis 5408$_{CIP}$	>200	>200	>200	>200	>200	>200
Enterococcus faecalis ATCC 29212	>200	**6.25**	200	>200	>200	>200
Staphylococcus aureus ATCC 25923	>200	>200	>200	>200	>200	>200
MRSA clinical strain	>200	>200	>200	>200	>200	>200
MRSA COL	>200	**50**	200	200	>200	200
MRSA COL$_{OXA}$	200	**25**	**100**	**50**	**100**	**100**
S. aureus HPV 107	>200	**25**	200	**50**	>200	**100**
Mycobacterium tuberculosis H37Rv	>200	**100**	>200	>200	200	>200

MGDG, monogalactosyldiacylglycerol; MRSA, meticillin-resistant *S. aureus.*

[a] See Section 2.2 for definition of strains.

[b] Significant MIC values (≤100 mg/L) are highlighted in bold.

Table 2

Effect of compounds isolated from *Carpobrotus edulis* on the minimum inhibitory concentrations (MICs) of tetracycline (TET) against *Escherichia coli* $AG100_{TET8}$ [a,b]

Strain	MIC (mg/L)											
	TET	TET + compound (mg/L)											
		β-Amyrin		Oleanolic acid		Uvaol		MGDG		Catechin		Epicatechin	
		50	100	50	100	50	100	50	100	50	100	50	100
E. coli $AG100_{TET8}$	25	12.5	25	**6.25**	**6.25**	12.5	**6.25**	12.5	12.5	25	**6.25**	12.5	**6.25**

MGDG, monogalactosyldiacylglycerol.

[a] *Escherichia coli* AG100 strain exposed to increasing concentrations of tetracycline leading to an efflux pump-overexpressing strain.

[b] Significant MIC values are highlighted in bold (≥4-fold reduction in MIC by a given compound).

Table 3

Effect of compounds isolated from *Carpobrotus edulis* on the minimum inhibitory concentrations (MICs) of ciprofloxacin (CIP) against *Salmonella enterica* serotype Enteritidis 5408_{CIP} and *S.* Enteritidis 104_{CIP} [a,b]

Strain	MIC (mg/L)											
	CIP	CIP + compound (mg/L)										
		β-Amyrin		Oleanolic acid		Uvaol		MGDG		Catechin		Epicatechin	
		50	100	50	100	50	100	50	100	50	100	50	100
S. Enteritidis 5408_{CIP}	>50	25	50	25	25	**6.25**	**6.25**	**6.25**	**12.5**	25	25	**12.5**	**12.5**
S. Enteritidis 104_{CIP}	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

MGDG, monogalactosyldiacylglycerol.

[a] *Salmonella* Enteritidis 5408 adapted to 16 mg/L ciprofloxacin and shown to have an overexpressed AcrB transporter.

[b] Significant MIC values are highlighted in bold (≥4-fold reduction in MIC by a given compound).

Table 4

Effect of compounds isolated from *Carpobrotus edulis* on the minimum inhibitory concentrations (MICs) of oxacillin (OXA) against MRSA COL_{OXA} [a,b]

Strain	MIC (mg/L)											
	OXA	OXA + compound (mg/L) [c]										
		β-Amyrin		Oleanolic acid	Uvaol		MGDG		Catechin		Epicatechin	
		50	100	10	25	50	12.5	25	25	50	25	50
MRSA COL_{OXA}	1600	1600	1600	1600	**<100**	**<100**	1600	1600	1600	1600	1600	1600

MRSA, meticillin-resistant *Staphylococcus aureus*; MGDG, monogalactosyldiacylglycerol.

[a] MRSA COL adapted to 1600 mg/L oxacillin.

[b] Significant MIC values are highlighted in bold (≥4-fold reduction in MIC by a given compound).

[c] Minimum concentration of OXA used was 100 mg/L and no growth was observed for uvaol. The results of the assay were read after 48 h when the strain control also grew.

Table 5

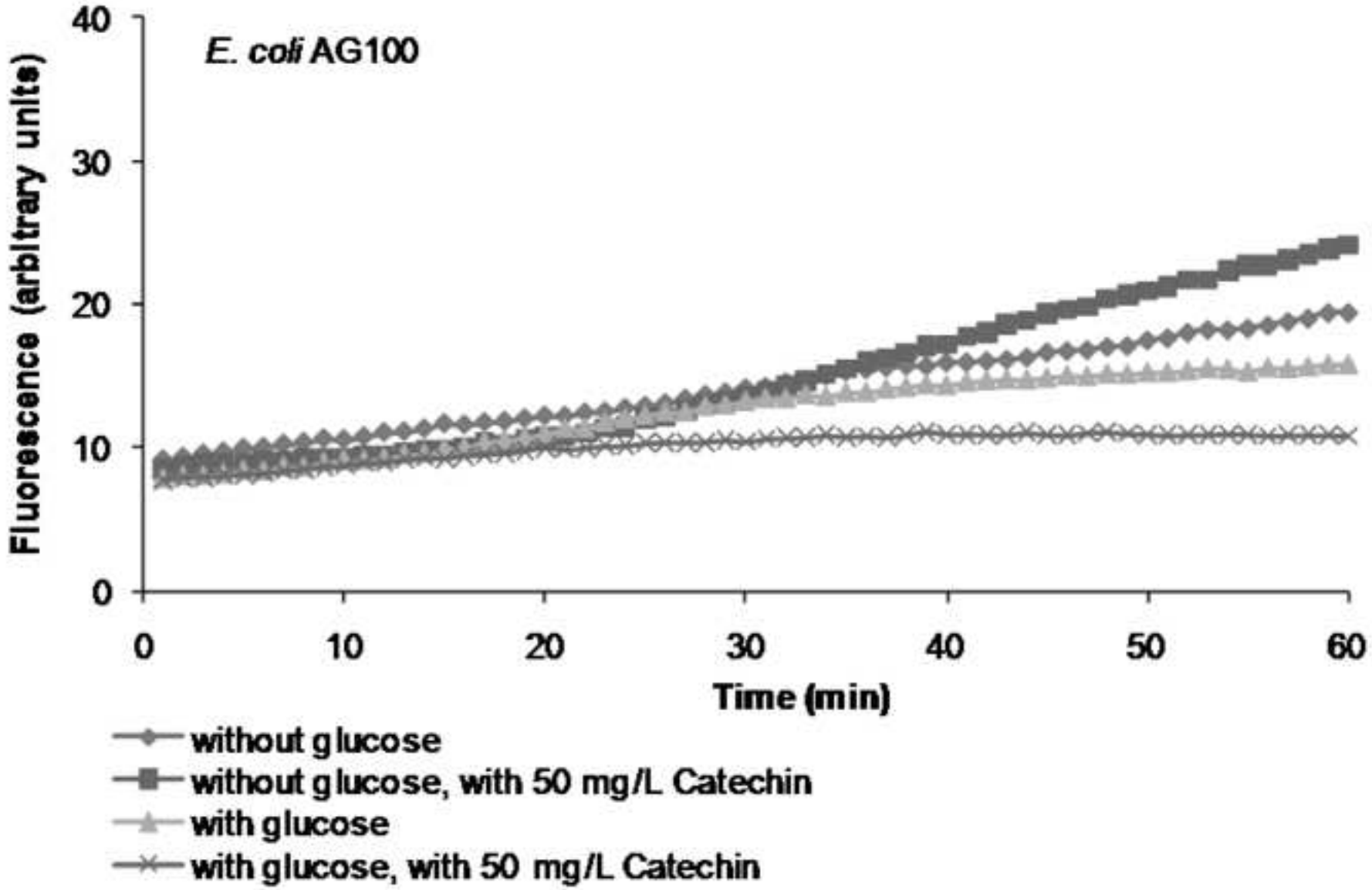
Effect of compounds isolated from *Carpobrotus edulis* on the minimum inhibitory concentrations (MICs) of oxacillin (OXA) against MRSA clinical strain [a]

Strain	MIC (mg/L)											
	OXA	OXA + compound (mg/L)											
		β-Amyrin		Oleanolic acid		Uvaol		MGDG		Catechin		Epicatechin	
		50	100	50	100	50	100	50	100	50	100	50	100
MRSA	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500

MRSA, meticillin-resistant *Staphylococcus aureus;* MGDG, monogalactosyldiacylglycerol.

[a] Significant MIC values are highlighted in bold (≥4-fold reduction in MIC by a given compound).

Edited Figure 1



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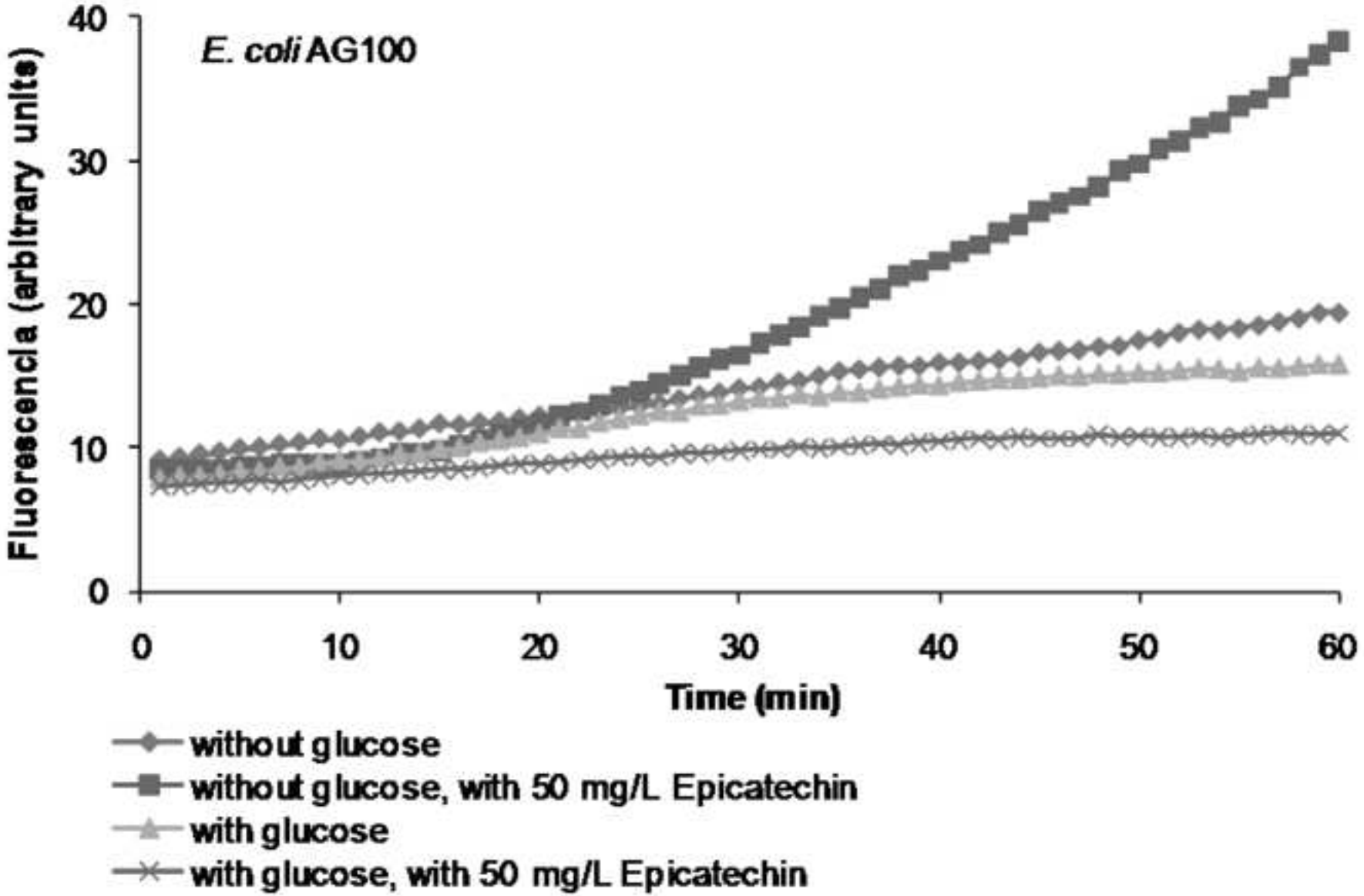
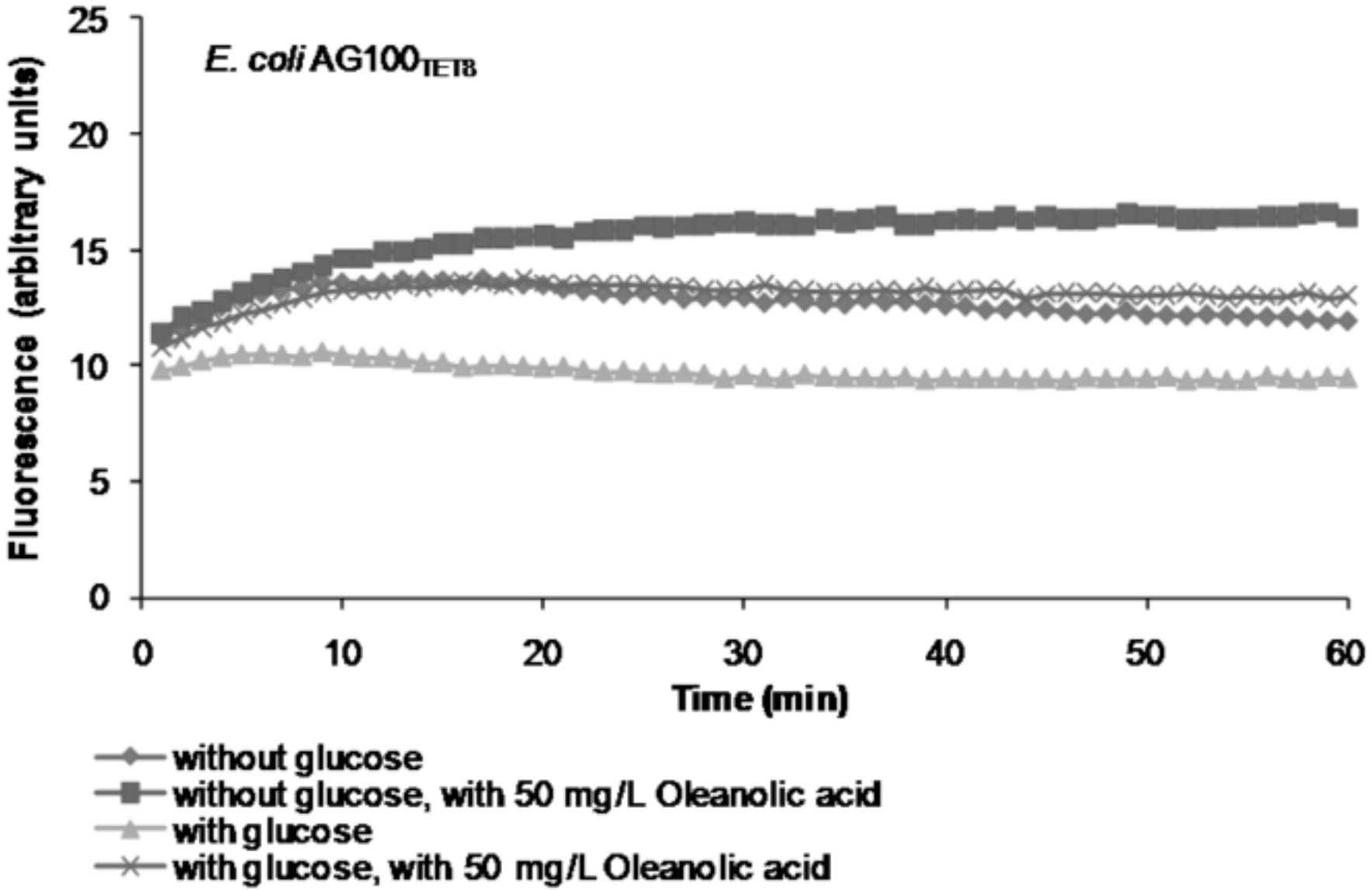


Edited Figure 3



Edited Figure 4