

# Antibacterial activity of oleanolic and ursolic acids and their derivatives

## Review Article

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**Abstract:** Bacterial resistance to antibiotics is increasing at an alarming rate and many commonly used antibiotics are no longer effective. Thus, there is considerable interest in investigating novel antibacterial compounds, such as the plant-derived pentacyclic triterpenoids, including oleanolic acid (OA), ursolic acid (UA) and their derivatives. These compounds can be isolated from many medicinal and crop plants and their antibacterial, antiviral, antiulcer and anti-inflammatory effects are well documented. OA and UA are active against many bacterial species, particularly Gram-positive species, including mycobacteria. They inhibit bacterial growth and survival, and the spectrum of minimal inhibitory concentration (MIC) values is very broad. In addition, OA, UA and their derivatives display potent antimutagenic activity. Studies to identify the cellular targets and molecular mechanisms of OA and UA action were initiated a few years ago and it has already been demonstrated that both acids influence bacterial gene expression, the formation and maintenance of biofilms, cell autolysis and peptidoglycan turnover. Before these compounds can be used clinically as antimicrobial agents, further extensive studies are required to determine their cytotoxicity and the optimum mode of their application.

**Keywords:** *Oleanolic acid • Ursolic acid • Antibacterial activity • Antimutagenic effect • Cellular targets*

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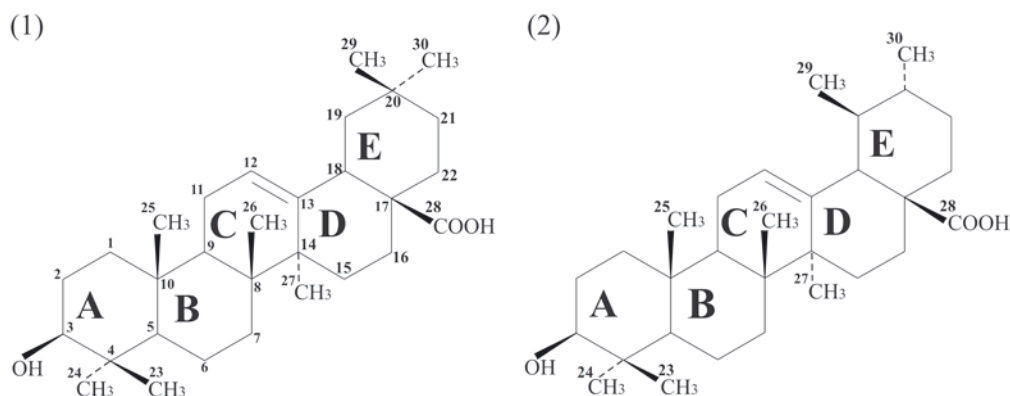
## 1. Introduction

Enormous quantities of antibiotics are currently produced and used to treat human and animal infections, added as supplements to feed in order to promote the growth of animals and also applied in agriculture to prevent microbial infections of cultivars [1]. Bacterial resistance to these agents is growing at an alarming rate so that many commonly used antibiotics are no longer effective. This dangerous situation urgently needs to be resolved and many approaches have been used to discover new and efficient “smart” antimicrobial drugs to counter the spread of resistance to current antibiotics [2]. There is also growing interest in studying alternative antimicrobial agents, e.g. bacteriophages [3], antibacterial peptides produced by microorganisms [4] and plant-derived compounds. The latter are listed and were reviewed by Cowan [5] more than a decade ago. There are several major groups of antimicrobial compounds of plant origin: phenolics and polyphenols (including simple phenols

and phenolic acids, quinines, flavones, flavonoids and flavonols, tannins and coumarins), terpenoids and essential oils, alkaloids, lectins and polypeptides.

Oleanolic acid (OA, olenane-type) and ursolic acid (UA, ursane-type) are representatives of the pentacyclic triterpenoids. These acids are based on the structure of isoprene and contain 30 carbon atoms and oxygen. Their chemical structures are shown in Figure 1. Both acids can acquire sugar moieties linked to the C-3 hydroxyl group of the aglycone, leading to the formation of the different types of glycosides, e.g. the glucosides (derivatives of 3-O-monoglucoside) and the glucuronides (derivatives of 3-O-monoglucuronide). Glucosidoesters and compounds containing other substitutions are also ubiquitous in the plant kingdom [6]. OA and UA are constituents of medicinal herbs and also form an integral part of the human diet [7]. Several approaches have been successfully used to improve their biological activity, diminish their toxicity [8] and enhance their water solubility [9].

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**Figure 1.** Structure of OA (1) and UA (2).

Herb mixtures are commonly used in traditional medicine and their clinical potential is well established. Many of these mixtures contain OA, UA or their derivatives. It is obvious that studies utilizing the purified compounds are necessary to determine the mechanisms of their biological action. Among the diverse activities of OA and UA, their anti-inflammatory [10], antiulcer [11], hepatoprotective [12], antidiabetic [13], fungicidal [14] and antiparasitic [15] effects are particularly notable. The antiviral potential of these compounds is also of special interest. OA, UA and their derivatives efficiently inhibit the development of several viruses including HIV. The mechanism of their antiviral activity is already partially resolved, which should permit the therapeutic use of these compounds in the near future [16-20].

A great deal of experimental data published in the last three decades have confirmed the potential of extracts of medicinal plants and several isolated compounds to cause growth inhibition of various bacterial genera, including multidrug resistant strains. However, other investigations found poor or negligible antibacterial activity. The majority of the studies have been performed using poorly defined bacterial strains and plant extracts. Furthermore, even those studies performed with purified compounds have often been restricted to the determination of MIC (minimal inhibitory concentration) or MBC (minimal bactericidal concentration) values (see chapters 2 and 3). Studies that have attempted to resolve the mechanisms underlying the efficacy of these compounds, or at least to identify the cellular targets of OA and UA, are described in the separate chapter.

This review on our current understanding of the antibacterial and antimutagenic activity of oleanolic and ursolic acids and their derivatives comprises reports published over the last 20 years. The publications cited are those presenting the best documented and most robust results. It should be mentioned that so far there are no rules recommended for the validation of OA

and UA antibacterial activity. According to Fontanay and coworkers [21] MICs values below  $10 \mu\text{g mL}^{-1}$  define “good” and those around  $50 \mu\text{g mL}^{-1}$  “moderate” antibacterial activity. MICs values that equal hundreds of  $\mu\text{g mL}^{-1}$  indicate that the compound is devoid of activity. The order of literature presented below follows the potential of the reported OA/UA antibacterial effects.

## 2. Antibacterial activity of OA and its derivatives

Bacterial species belonging to the genus *Mycobacterium* are supposed to be sensitive to triterpenoids, including OA and UA, because of the high sterol content of their cell envelopes [22]. Due to the rapid emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*, there is an urgent need to discover a new therapeutics for the treatment of tuberculosis [23]. The antimycobacterial activity of five plant species used in the traditional medicine in South Africa was studied by Bamuamba and coworkers [24] (Table 1). OA present in the extracts of *Buddleja saliga* (*Buddlejaceae*) and *Leysera gnaphalodes* (*Asteraceae*) exhibited a significant antimycobacterial effect. MIC values for *M. tuberculosis* H37RV, *Mycobacterium avium*, *Mycobacterium serofulaceum* and *Mycobacterium microti* were between  $1.25 \mu\text{g mL}^{-1}$  and  $2.5 \mu\text{g mL}^{-1}$ , as determined by the bioautography method based on the MTT colorimetric assay [25]. The fact that OA showed no toxicity to Chinese hamster ovary (CHO) cells added considerably to the pharmaceutical value of these compounds. Subsequently it was demonstrated that the modification of OA to produce *p*-coumarate ester analogue resulted in a substantial, eight-fold increase in its antibacterial potential suggesting a strong structure–activity relationship

[26]. In turn Rojas and coworkers demonstrated that the OA derivative, aegicerin, extracted from the Peruvian plant *Clavija procera* (*Theophrastaceae*) possessed very strong activity against a large number of *M. tuberculosis* strains, including clinical isolates resistant to isoniazid and the multidrug-resistant (MDR) isolates. MIC values ranged between 1.6  $\mu\text{g mL}^{-1}$  and 3.12  $\mu\text{g mL}^{-1}$  [27]. From the clinical perspective, very important results were obtained by Horiuchi and coworkers who reported the strong activity of OA and UA isolated from *Salvia officinalis* (*Lamiaceae*) extract against VRE (vancomycin-resistant enterococci) with MIC value of 8  $\mu\text{g mL}^{-1}$  [28]. This result was confirmed for the reference strain, *Enterococcus faecalis* ATCC 28212 [21] (Table 1). Olean-27-carboxylic acid-type triterpenes extracted from the root portion of *Aceriphyllum rossi* (*Saxifragaceae*) which is a staple food in Korea, exhibited potent antibacterial activity against several strains of MRSA (methicillin-resistant *S. aureus*)

and quinolone-resistant *Staphylococcus aureus* [29]. This result indicated that both the carboxylic group at C-27 and the hydroxyl group at C-24 in aceriphylllic acid A are critical for the strong bactericidal activity. OA isolated from the Argentinian legume *Caesalpinia paraguayensis* (*Fabiaceae*) exhibited good activity against *Bacillus subtilis*, with MICs of 8  $\mu\text{g mL}^{-1}$  [30] (Table 1). A recent study from our group demonstrated that OA isolated from marigold, *Calendula officinalis* (*Asteraceae*) was active against many bacterial species, especially *Staphylococcus epidermidis* (Table 1). Oleanolic acid was also shown to be more active than its 3-O-monoglucoside and other glucosides and glucuronides, indicating that the aglycone structure appears necessary and sufficient for antibacterial activity [31].

All other species studied by Szakiel and coworkers: *Listeria monocytogenes*, *Bacillus megaterium*, *Shigella flexneri*, *Shigella sonnei*, *Yersinia enterocolitica* O:8

Compound	Potential of activity	Bacterial strain	MIC value $\mu\text{g mL}^{-1}$	Reference
OA	Good	<i>M. tuberculosis</i> H37Rv	2.5	[24]
		VRE	8	[28]
		<i>E. faecalis</i> ATCC 28212	8	[21]
		<i>B. subtilis</i>	8	[30]
		<i>S. epidermidis</i>	10	[31]
	Moderate	<i>L. monocytogenes</i>	15	[31]
		<i>B. megaterium</i>	35	[31]
		<i>S. flexnerii</i>	50	[31]
		<i>S. sonnei</i>	55	[31]
		<i>Y. enterocolitica</i> O:8	60	[31]
		<i>M. tuberculosis</i> H37Rv	30	[33]
		<i>M. tuberculosis</i> H37Rv	50	[36]
		MRSA	64	[30]
	Poor or none	<i>S. aureus</i> ATCC 25923	64	[21]
		<i>K. pneumoniae</i>	100	[31]
		<i>E. coli</i>	156	[44]
		<i>P. aeruginosa</i>	>256	[21]
		<i>P. gingivalis</i>	625	[45]
<i>A. viscosus</i>		625	[45]	
<i>P. intermedia</i>		1250	[45]	
UA	Good	<i>M. tuberculosis</i> H37Rv	2.5	[24]
		MRSA	3	[53]
		VRE	4	[28]
		<i>S. aureus</i> ATCC 25923	8	[21]
	Moderate	<i>B. subtilis</i>	25	[55]
		<i>P. syringae</i>	25	[55]
		<i>B. sphaericus</i>	50	[55]
		<i>E. coli</i>	50	[55]
		<i>S. typhi</i>	50	[55]
		<i>M. tuberculosis</i> H37Rv	31	[33]
Poor or none	<i>M. tuberculosis</i> H37Rv	65	[60]	
	<i>H. pylori</i> ATCC 43504	50	[63]	
	Poor or none	<i>P. aeruginosa</i>	>256	[21]

**Table 1.** Antibacterial activity of OA and UA.

VRE – vancomycin-resistant enterococci; MRSA – methicillin-resistant *S. aureus*.

and *Escherichia coli* were moderately susceptible to OA [31] (Table 1). Gu and coworkers evaluated the antitubercular activity of OA extracted from *Quinchamalium majus* (Santalaceae), using the microplate alarm blue assay (MABA) system [32] and discussed structure–activity relationships. They showed that OA was active against *M. tuberculosis* with a MIC of 30  $\mu\text{g mL}^{-1}$ . They also found that the presence of a hydroxyl group in the A ring combined with a carboxylic group in the E ring (see Figure 1) was associated with the observed antitubercular activity [33] (Table 1). The same group showed that OA isolated from *Valeriana laxiflora* (Valerianaceae) was responsible for the antitubercular effect of this plant extract [34]. It was also demonstrated that OA from *Lantana hispida* (Verbanaceae) was active against *M. tuberculosis* H37Rv and its streptomycin-, isoniazid-, rifampin- and ethambutanol-resistant variants, as determined by MABA. MIC values were 25  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$  for H37Rv and drug-resistant variants, respectively [35]. Caldwell and coworkers showed that OA isolated from Argentinean shrub, *Junella tridens* (Verbanaceae) was active against *M. tuberculosis* H37Rv [36]. The MIC of OA determined using a BACTEC radiospirometric assay [37] was 50  $\mu\text{g mL}^{-1}$  (Table 1). OA-derivatives, oleanonic acid and 3-*epi*-oleanonic acid, were three-fold more active. OA extracted from *Caesalpinia paraguarensis* (Fabaceae) displayed moderate activity against methicillin-sensitive *S. aureus* and MRSA strains with MICs of 64  $\mu\text{g mL}^{-1}$  [30] as determined by the microbroth dilution method [38] recommended by CLSI [39]. The same result was obtained for the *S. aureus* ATCC 25923 reference strain [21] (Table 1). Scalón Cunha and coworkers evaluated the antimicrobial activity of OA isolated from *Miconia* species (Melastomaceae) against the following microorganisms: *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus salivarius* and *E. faecalis*, which are potentially responsible for the formation of dental caries and found that this compound exerted an moderate antibacterial effect with MIC values ranging from 30  $\mu\text{g mL}^{-1}$  to 80  $\mu\text{g mL}^{-1}$  [40]. With regard to the structure–activity relationship of triterpene acids and their derivatives, it was suggested that both hydroxy and carboxy groups present in triterpenes are important for their antibacterial activity against oral pathogens. The substantial growth inhibition of some phytopathogenic bacteria by hederagenin isolated from *Medicago sativa* (Leguminosae) [41] and by other saponins – OA-derivatives, isolated from *Lepidagathis hyaline* (Acanthaceae) and *Lacuta scariola* (Plantaginaceae) were also reported [42,43].

Several reports have failed to demonstrate the antibacterial potential of OA. Poor activity of OA against

*Klebsiella pneumoniae* [31], *E. coli* and other Gram-negative bacteria [44], and several clinical isolates of *Pseudomonas aeruginosa* [21] have been reported (Table 1). Cai and Wu were unable to identify any activity of OA isolated from a methanol extract of the clove *Syzygium aromaticum* (Myrtaceae) against the Gram-negative anaerobic periodontal pathogens *Porphyromonas gingivalis* and *Prevotella intermedia* or the Gram-positive cariogenic oral bacteria *S. mutans* and *Actinomyces viscosus* [45]. The reported MIC values were in the range of 625–1250  $\mu\text{g mL}^{-1}$  (Table 1). Likewise, OA isolated from *Syzygium guineense* (Myrtaceae) did not inhibit the growth of *E. coli*, *S. sonnei* or *B. subtilis*, as estimated using bioautography on thin-layer plates [46]. In a paper by Panizzi and coworkers it was shown that OA isolated from an extract of *Geum rivale* (Rosaceae) was not active against *S. aureus*, *E. coli* or *P. aeruginosa* [47], as determined by quantitative screening of bacterial number using the agar well diffusion assay [48]. Wächter and coworkers demonstrated poor antitubercular activity of OA-derivatives isolated from *Lippia turbinata* (Verbenaceae), with MIC values varying from 64  $\mu\text{g mL}^{-1}$  to 128  $\mu\text{g mL}^{-1}$  [49]. Caliş and coworkers did not observe any antimicrobial activity of OA isolated from *Cyclamen mirabile* (Primulaceae) against Gram-positive (*S. aureus* and *E. faecalis*) and Gram-negative (*P. aeruginosa* and *E. coli*) species [50]. The antimicrobial constituents of *Gentianopsis paludosa* (Gentianaceae), the plant used in local Tibetan medicine to treat pneumonia, diarrhea and several other diseases were recently investigated and found to include OA, which when purified, was inactive against *Mycobacterium smegmatis* (MIC>500  $\mu\text{g mL}^{-1}$ ) and *M. tuberculosis* (MIC>128  $\mu\text{g mL}^{-1}$ ) [51].

### 3. Antibacterial activity of UA and its derivatives

The data published in the previously cited paper of Bamuamba and coworkers demonstrated the potent antimycobacterial activity of UA present in the extract of *Leysera gnaphaloides* which was comparable with the activity of OA and fluctuated between 1.25  $\mu\text{g mL}^{-1}$  and 2.5  $\mu\text{g mL}^{-1}$  [24] (Table 1). In another paper 99% inhibition of *M. tuberculosis* growth by UA extracted from *Chamaedora tepejilote* (Palmae) was reported [52]. It was also demonstrated that UA purified from *Baccharis dracunculifolia* (Asteraceae), which is the most important plant source of Brazilian green propolis, possessed strong antibacterial activity against MRSA [53] (Table 1). In turn Horiuchi and coworkers

reported the potent antibacterial activity of UA isolated from *S. officinalis* against VRE, with a MIC value of  $4 \mu\text{g mL}^{-1}$  [28] and Fontanay and coworkers proved the substantial activity of UA against *S. aureus* – MIC  $8 \mu\text{g mL}^{-1}$  [21] (Table 1). Strong inhibition of *S. aureus* growth by ursolate glycosides:  $\beta$ -gentabioside and  $\beta$ -maltotrioside has been demonstrated [54], and the potent antimycobacterial activity of 3-*epi*-ursolic acid isolated from *Calceolaria pinnifolia* (*Scrophulariaceae*) has also been described [55].

UA extracted from *Diopsyros melanoxylo*n (*Ebenaceae*) and its lipophilic 3-O fatty acids ester chains ( $\text{C}_{12}$ – $\text{C}_{18}$ ) were evaluated for antimicrobial activity against *Pseudomonas syringae*, *Bacillus sphaericus*, *Bacillus subtilis*, *E. coli* and *Salmonella typhi* and moderate activity was reported [56] (Table 1). Also Chattopadhyay and coworkers found that *n*-butanol extracts of *Alstonia macrophylla* (*Apocyanaceae*) [57] and *Mallotus pelatus* (*Euphorbiaceae*) [58], enriched in UA and used in South Asian traditional medicine, showed moderate antibacterial activity against *Staphylococcus aureus*, *Staphylococcus saprophytus*, *Streptococcus faecalis*, *B. subtilis* and *Proteus mirabilis*. The antibacterial activity of an extract of *G. rivale* and isolated compounds including UA was moderately active against *S. aureus*, *P. aeruginosa* and *E. coli* [47]. Antibacterial activity against eight bacterial species was also reported for UA and its derivatives isolated from whole plants of *Rostellularia procumbens* (*Acientaceae*). At the same time no cytotoxic activity against HCT-8 and Bell-7402 cell lines was observed [59]. UA extracted from *Q. majus* was shown to possess moderate antitubercular activity with a MIC of  $31 \mu\text{g mL}^{-1}$  [33] (Table 1). The antimycobacterial activity of UA against *M. tuberculosis* H37Rv and its derivative – the fluorescent (GFP) strain H37Rv-pFPCA1 was studied by Jaki and coworkers [60] using MABA and the green fluorescent protein microplate assay (GFPMA) [61]. The reported MIC values were quite high –  $65 \mu\text{g mL}^{-1}$  for an 81%-pure sample (Table 1). It was also demonstrated that ursolic-3-*p*-coumarate extracted from *Piliglostigma tropicum* (*Myrtaceae*) possessed broad antibacterial activity [62]. Recently, due to its acid-neutralizing and antioxidant activities, UA isolated from *Gardenia jasminoides* (*Rubiaceae*) was shown to be a potential inhibitor of *Helicobacter pylori* growth when applied in a concentration of  $50 \mu\text{g mL}^{-1}$  [63] (Table 1). Therefore, this compound could be a useful therapeutic agent for the treatment of and/or protection against gastritis.

Very poor or negligible antibacterial activity against Gram-positive and Gram-negative species, including *S. aureus*, *B. subtilis* and *E. coli*, was demonstrated for UA isolated from *Debregeasia salicifolia* (*Virticaceae*) [64],

*Vitex negundo* (*Verbenaceae*) [65] and *Ligularia sagitta* (*Astraceae*) [66]. Similarly, no antibacterial activity against MRSA was found for UA isolated from *Rosa nutkana* (*Rosaceae*) [67]. Also Fontanay and coworkers did not report UA activity against *P. aeruginosa* [21].

The reason for the contrasting data, indicating either a strong or weak antimicrobial effect of UA (and also OA), was discussed by Jaki and coworkers [60]. They explored the variability of the biological responses to these compounds from the perspective of the sample purity–activity relationships (PAR), focusing on the activity of UA against *M. tuberculosis*. They concluded that increasing the purity reduced the biological activity. However this explanation is not entirely satisfactory since potent antibacterial activity was observed using commercially available, very pure stocks of OA and UA. The differences are more likely to result from the method of compound purification and the bacterial strains studied.

#### 4. Antimutagenic activity of OA and UA and their derivatives

OA and UA not only influence bacterial growth but also possess antimutagenic activity. Niikawa and coworkers showed that OA and UA isolated from the extract of *Ligustrum lucidum* (*Oleaceae*) inhibited the mutagenic activity of benzopyrene towards *Salmonella enterica* sv. Typhimurium TA98 [68]. The inhibition of the benzo[ $\alpha$ ]-pyrene was evaluated with modification of the Ames test [69] and OA/UA concentration used ( $1 \text{ mg/plate}$ ). This suppressed reversion frequency by 95% did not influence bacterial growth. The study by Lira Wde and coworkers confirmed the antimutagenic potential of OA and UA [70]. Both compounds extracted from *Byrsonima basiloba* (*Malpighiaceae*), when used in the sublethal concentrations, suppressed the number of revertants, as was evaluated in the Ames test using four *S. enterica* sv. Typhimurium strains (TA97a, TA98, TA100 and TA102) and direct or indirect mutagenic agents, such as 4-nitro-*o*-phenylenediamine, sodium azide, mitomycin C and aflatoxin B [70]. It was also shown that UA isolated from an ethanol extract of *Uncaria sinensis* (*Rubiaceae*) diminished aflatoxin B1-induced mutagenicity in *S. enterica* sv. Typhimurium. The number of revertants decreased three-fold at UA concentration not toxic for bacteria ( $500 \mu\text{g/plate}$ ) [71]. Another report demonstrated the suppression of mutagenic activity of heterocyclic amine (Trp-P-1) – a DNA damaging agent, by UA isolated from *Uncaria sinensis* (*Rubiaceae*) [72]. In this study the Umu-

dependent mutagenesis, being the part of SOS response system induced by DNA damage, was explored [73]. UA in concentration  $0.4 \mu\text{mol ml}^{-1}$  ( $\text{ID}_{50}=0.17 \mu\text{mol ml}^{-1}$ ) reduced Trp-P-1-induced *umuC* gene expression and therefore SOS-induced mutagenesis by 61.3%. Like OA and UA their derivatives also possess antimutagenic activity. Ohtsuka and coworkers reported the two-fold inhibition of 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide mutagenicity by ginsenosides isolated from the Chinese medicinal herb sho-saiko-to and applied in concentrations of 2 mg/plate which is not toxic for *S. enterica* sv. Typhimurium TA100 [74]. Lee and coworkers demonstrated the antimutagenic effect of hederagenin monodesmosides and bidesmosides, kalopanaxaponin A and kalopanaxsaponin B and H, extracted from *Kalopanax pictus* (*Araliaceae*). These compounds diminished aflatoxin B<sub>1</sub> (AFB<sub>1</sub>)-induced mutagenesis but had no effect on mutagenesis caused by *N*-methyl-*N'*-nitro *N*-nitrosoguanidine (MNNG). This suggested that hederagenin and its 3-*O*-glycosides might efficiently prevent the metabolic activation of AFB<sub>1</sub> or scavenge the electrophilic intermediate capable of inducing mutation [75]. It was also shown that the mutagenic potential of aflatoxin B<sub>1</sub> could be inhibited by kaikasaponin III and tectorigenin from *Pueraria thumbergiana* (*Leguminosae*). Kaikasaponin III (1 mg/plate) decreased the number of *S. typhimurium* TA100 revertants induced by AFB<sub>1</sub> by 99% and those induced by MNNG by 75%. Tectorigenin (1 mg/plate) inhibited AFB<sub>1</sub>-induced mutagenicity by 90% and MNNG-induced mutagenicity by 76%. Compound concentrations employed for the antimutagenic test did not show any toxicity for the test strain [76].

The lack of OA antimutagenic potential was also reported. This compound was found to be unable to inhibit UV-induced formation of 8-hydroxy-2-deoxyguanosine (8-OHdG), which causes oxidative DNA damage. The reason for this effect lies in the inability of OA to intercalate into DNA, as shown by computer modeling [77].

## 5. Cellular targets and functions affected by OA, UA and their derivatives

A small number of studies have investigated the basis of antibacterial activity of OA, UA and their derivatives at a subcellular level. The previously described results of Szakiel and coworkers [31] demonstrated that OA not only acted as a potent inhibitor of the growth and survival of several species but it also influenced bacterial shape. *E. coli* cells appeared several-fold longer, in contrast *B. megaterium* cells became shorter after an overnight incubation with OA at a concentration of  $0.7 \times \text{MIC}$ . Also, OA affected sporulation of *B. megaterium* and enhanced Triton X-100 induced lysis of this species. The final study suggests the eventual stimulation of murein hydrolases [78] by OA can result in increased degradation of this macromolecule. A detailed study of the effects of OA and UA on peptidoglycan (murein) content, turnover and the autolysis of *L. monocytogenes* was published recently [79]. In this species, the causative agent of listeriosis is a facultative pathogen able to survive and multiply in macrophages [80]. Members of the genus *Listeria* are still sensitive to a wide range of antibiotics, although the emergence of antibiotic resistance has been reported [81]. In the study by Kurek and coworkers on *L. monocytogenes* peptidoglycan, it was confirmed that the application of chemically pure commercial preparations of both acids in concentration  $0.7 \times \text{MIC}$  influenced cell morphology and enhanced detergent-induced lysis of this bacterium. It was also shown that the autolysis of isolated cell walls was inhibited by OA and UA, although this effect was not very pronounced. OA and UA, in a concentration of  $0.5 \times \text{MIC}$ , inhibited peptidoglycan turnover and quantitatively influenced the profile of mucopeptides obtained by the digestion of peptidoglycan with mutanolysis and compared

Compound	Bacterial strain	Cellular target/affected function	Reference
OA	<i>L. monocytogenes</i> EGD	Peptidoglycan metabolism, probably peptidoglycan hydrolases	[79]
OA-CDs	<i>S. mutans</i> MT81148R	Isg synthesis	[82]
Glycyrrhizin	ETEC	$\beta$ -subunit of LT enterotoxin	[83]
UA	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>V. harveyi</i>	Biofilm formation and maintenance	[85]
UA	<i>E. coli</i>	Differential gene expression	[85]

**Table 2.** Cellular targets and functions affected by OA and their derivatives and UA.

OA-CDs – oleanolic acid-cyclodextrins; ISG – insoluble glucan; ETEC – enterotoxigenic *E. coli*.

to the control, the monomer and dimer fractions were increased. As both OA and UA fail to bind to *L. monocytogenes* PBPs (penicillin binding proteins) involved in the late stage of cell wall synthesis, peptidoglycan hydrolases are the most probable targets of these pentacyclic triterpenoids (Table 2).

In a report published 10 years ago, Kozai and coworkers showed that two oleanolic acid cyclodextrin inclusion compounds (OA-CDs), OA-G1- $\beta$  CD and OA- $\beta$  CD, inhibited insoluble glucan (ISG) synthesis by *S. mutans* MT81148R (Table 2). Examination of the anticariogenic effect of OA- $\beta$  CD in the rat caries model indicated that this compound could be a potential anticaries agent [82].

Chen and coworkers, in their recent studies, applied *in silico*, *in vitro* and *in vivo* analyses to show that olean-type triterpenoid, glycyrrhizin, present in *Glycyrrhiza uralensis* (Fabaceae) can act as a potent *E. coli* enterotoxin inhibitor when applied in concentrations higher than 4 mM [83]. Heat-labile enterotoxin (LT) is the major virulence factor of ETEC (enterotoxigenic *E. coli*) [84], and the glycyrrhizin treatment significantly reduced the ability of the  $\beta$ -subunit of LT to bind to the surface of intestinal epithelial cells, as shown by  $G_{M1}$ -ELISA and also suppressed LT-induced fluid accumulation in mice (Table 2).

In a landmark study, Ren and coworkers demonstrated that UA caused differential gene expression in *E. coli* and inhibited biofilm formation in several bacterial species (Table 2) [85]. In these studies UA was applied at sublethal concentrations, up to 30  $\mu\text{g mL}^{-1}$ , which did not inhibit the growth of any species tested. For example, the specific growth rate of *E. coli* K-12 ATCC 25404 was  $1.29 \pm 0.12 \text{ h}^{-1}$  without ursolic acid,  $1.36 \pm 0.07 \text{ h}^{-1}$  with ursolic acid at 10  $\mu\text{g mL}^{-1}$  and  $1.16 \pm 0.08 \text{ h}^{-1}$  with ursolic acid at 30  $\mu\text{g mL}^{-1}$ . It was shown using the crystal violet of biofilm staining method [86] that UA at a concentration 10  $\mu\text{g mL}^{-1}$  inhibited biofilm formation by 87% for *P. aeruginosa* PAO1, by 79% for *E. coli* K-12 ATCC 25404 and by 57% for *Vibrio harveyi* BB120. Ursolic acid inhibited biofilms without interfering with quorum sensing, as shown with the *V. harveyi* Al-1 and Al-2 reporter systems. This observation is of tremendous potential for medical value since biofilms are important pathogenicity determinants of many bacterial species [87]. The influence of UA on *E. coli* K-12 gene expression was studied using the DNA micro-array technique [88]. It was shown that this compound consistently and significantly ( $P < 0.05$ ) induced 19 genes which function in chemotaxis and motility (*cheA*, *tap*, *tar*, *motAB*), and the heat shock response (*hslSTV* and *mopAB*). Since the coordinated regulation of chemotaxis and motility gene expression

is important for biofilm formation and maturation [89], it was argued that the induction of these genes at the wrong stage of biofilm development may prevent its formation and/or destabilize the mature biofilm. Studies of the complete transcriptome also showed that UA consistently repressed 12 genes including those involved in cysteine synthesis (*cysK* and *cysB*), which might constitute another factor indirectly affecting biofilm formation [85]. The number of publications on the stability, cytotoxicity and mode of application of OA and UA is also not very substantial. In several reports the lack of OA/UA cytotoxic effect was described [for example 24,59,90] however contradictory information was also published [21,91]. Moreover special interest is paid to the synthesis of their derivatives with less toxicity [8]. Research aimed to improve the bioavailability and biological effect of these triterpenoids, which water solubility is limited, are also performed. Efforts have been made to improve their water solubility with chemically modified derivatives [7]. The synthesis of a non-covalent complex with hydrophilic cyclodextrins, as well as the use of nanosuspensions, enhancing both, solubility and stability (lipid emulsion containing corn oil or applying a self-nano-emulsified drug delivery system), are of special interest [9,92,93].

## 6. Conclusions

Increasing bacterial resistance to antibiotics has intensified the search for alternative therapeutic agents that may include secondary plant metabolites such as triterpenoids. Two pentacyclic triterpenoids, OA and UA, show appreciable antibacterial activity. The spectrum of MIC values is very broad and their activity depends mainly on the species (strain) tested. Few studies have attempted to identify the cellular targets and the mechanisms of OA and UA activity. It has been shown that these compounds influence peptidoglycan structure and composition, gene expression and biofilm formation. However, before the clinical application of OA and UA as alternatives to antibiotics, further extensive studies are required to resolve the molecular basis of their activity and to precisely determine their potential cytotoxicity.

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