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Effect of asiatic and ursolic acids on growth and virulence factors of uropathogenic *Escherichia coli* strains

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Abstract: This work was aimed at determining the possible antimicrobial potential of asiatic acid (AA) and ursolic acid (UA) against clinical uropathogenic *Escherichia coli* strains (UPECs). Phylogenetic affiliations of UPECs and virulence-associated gene carriage have been identified. PCR analysis revealed that the studied *E. coli* strains belong to phylogenetic group B2. All bacterial isolates were grouped into 4 pathotypes. The following were determined: the minimum inhibitory concentrations (MICs) according to CLSI; P-fimbriae synthesis, based on erythrocyte agglutination; curli fibers, by using YESCA agar with congo red; swimming motility, in motility agar; and alpha-hemolysin, on sheep's-blood agar plates. The differences in antigrowth activities of AA and UA were recorded. Inhibition of P-fimbriae, curli fibers, and alpha-hemolysin production after exposure of UPECs to both AA and UA were observed. Reduction of bacterial motility was also noticed. Our results clearly show the influence of AA and UA on virulence factors of UPEC strains. However, differences between antivirulence activities of AA and UA were not found, although a higher level of antigrowth activity of AA in comparison with UA was shown.

Key words: Uropathogenic *Escherichia coli*, asiatic acid, ursolic acid, minimum inhibitory concentration, alpha-hemolysin, curli fibers, P-fimbriae, motility

1. Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases in the human population. Bacteria are the causative agents responsible for more than 95% of UTIs. Most of these infections are caused by uropathogenic *Escherichia coli* strains UPECs (Foxman, 2010). Several potential virulence factors including adhesins, motility, and hemolysins may be responsible for the pathogenicity of UPECs. P-fimbriae and curli fibers promote UPEC adhesion, which is essential in colonization of the host tissues during the initial stages of an infection. P-fimbriae are the most prevalent fimbrial type of UPEC strains causing pyelonephritis (Stapleton, 2005). The colonization of the urinary system epithelium requires penetration of the mucus layer; therefore, the ability to move is a very useful feature of UPECs that facilitates infection. These bacteria also produce a pore-forming alpha-hemolysin, which is an important factor in causing necrosis of the eukaryotic cells. Many people suffer from recurrent UTIs; therefore, repeated treatment with antibiotics may lead to increased resistance of bacteria (Gupta et al., 2001; Foxman, 2010). To overcome the problem of antibiotic resistance, medicinal plants have been intensively studied as alternative or supplementary treatment methods against bacterial diseases (Khan et al., 2009; Mavi et al., 2011; Teyeb et al., 2011; Baykan Erel et al., 2012; Pehlivan Karakaş et al., 2012). Among various active substances present in plants, pentacyclic triterpenes display antibacterial properties (Chung et al., 2011).

Pentacyclic triterpenes are a group of natural compounds widespread in the plant kingdom (Jager et al., 2009). Asiatic acid (AA) and ursolic acid (UA) possess similar chemical structures (Figure 1); both belong to the aglycone pentacyclic triterpenes. AA is an important component of the medicinal plant *Centella asiatica* (James and Dubery, 2009). UA is found in many common plants (Ikeda et al., 2008; Yamaguchi et al., 2008). Many reports describe the antiinflammatory, hepatoprotective, gastroprotective, antiulcer, anti-HIV, cardiovascular, hypolipidemic, antiatherosclerotic, and immunoregulatory activities of AA and UA (Cho et al., 2006; Jeong et al., 2007; Ikeda et al., 2008). Despite these reports, their antimicrobial properties have been poorly investigated. Available reports mainly describe the activities of pentacyclic triterpene-containing plant extracts against gram-positive bacteria (Kurek et al., 2010;

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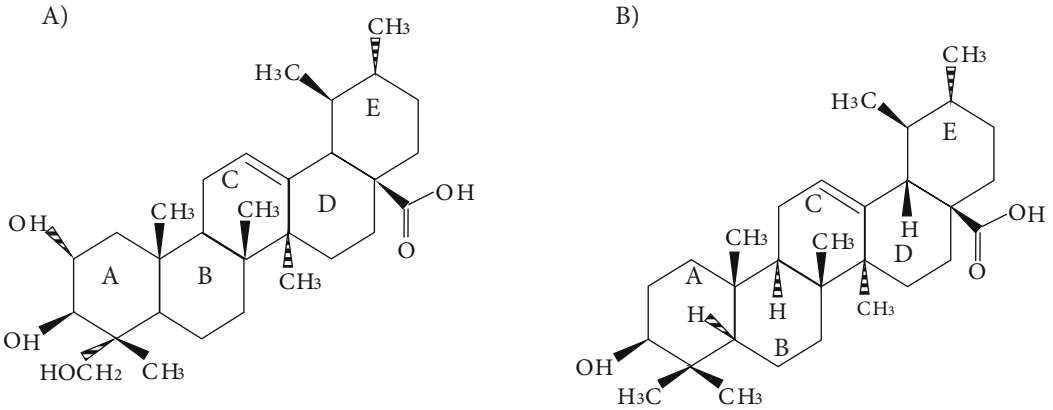

Figure 1. Chemical structures of AA (A) and UA (B). A, B, C, D, E – the names of rings in the molecules.

Chung et al., 2011). To our knowledge, reports describing the antibacterial activities of pentacyclic triterpenes, especially against UPECs, have been very limited; this fact prompted us to perform the current study.

The aim of our study was to evaluate the antimicrobial activities of AA and UA and their effect on growth, P-fimbriae, curli fibers, alpha-hemolysin synthesis, and swimming motility of UPEC strains. We also made an attempt to determine the correlation between the acids' structure and their impact on the survival and virulence factors of tested *E. coli* rods.

2. Materials and methods

2.1. Bacterial strains

Twenty *E. coli* clinical strains were isolated from the urine of patients suffering from pyelonephritis who were hospitalized in the Academic Clinical Centre of Wroclaw Medical University. The biochemical species affiliation of the examined strains was confirmed using the API-20E test kit (BioMérieux, Poland). The strains were maintained on Mueller-Hinton agar slopes (Oxoid) at 4 °C.

2.2. Phylogenetic classification and virulence-associated gene carriage

The detection of specific nucleotide sequences was confirmed by PCR on the total bacterial DNA using the GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit (EURx, Poland). All PCR analyses were performed using DreamTaq DNA polymerase (Fermentas, Germany). Membership of studied strains in a certain phylogenetic group was determined using primers specific for 2 genes (*chuA* and *yjaA*) and a DNA fragment (*TspE4.C2*), according to the methods of Clermont et al. (2000). PCR assays revealed that 20 *E. coli* isolates fell into phylogenetic group B2. Studied strains were also screened for the presence of genes encoding adhesins (*papC*, *afa*, *draE*, *csgA*) and a toxin (*hlyA*). Sequence coding for 16S rRNA was used as a positive control. The characteristics of all used primers, except *draE* primers, as well as amplicon lengths, were listed in our previous study (Wojnicz et al., 2012). Primers 5'-GACAGTTTACTGATTCTGGGA-3' and 5'-ACGTCCAATAATGCTTACCC-3' were used in the PCR analysis to amplify the *draE* gene (Matar et al., 2005). The presence of PCR-amplified DNA fragments was confirmed in 2% agarose gel. Gel images were analyzed using the Quantity One System (Bio-Rad, USA).

2.3. Antimicrobial agents

AA (purity of ≥97%) and UA (purity of ≥90%) were purchased from Sigma-Aldrich (Poland). Both acids were dissolved in 96% ethanol (heated to 70 °C) to obtain 10 mg/mL stock solutions. Final concentrations of AA and UA were prepared from the stock solutions using Mueller-Hinton broth (MHB).

2.4. MIC assay

All strains were grown in MHB at 37 °C overnight, resulting in approximately 10^8 CFU/mL. The minimum inhibitory concentrations (MICs) of AA and UA were determined by the broth microdilution method (CLSI, 2008). Briefly, stock solutions of triterpenes (10 mg/mL) were dissolved in MHB to obtain concentrations ranging from 4 to 1024 µg/mL, and then 200 µL of each concentration was added to each well of a 96-well microplate and incubated with the examined bacterial strain at 37 °C for 24 h. The lowest concentration of acid that inhibited bacterial growth was defined as the MIC. Each assay was repeated 3 times.

2.5. Effect of AA and UA on bacterial growth

The bacteria were grown overnight at 37 °C in MHB. Next, bacteria were centrifuged and resuspended in phosphate-buffered saline (PBS) to obtain a concentration of approximately 10^8 CFU/mL. The 10 µL of bacterial suspension was mixed with appropriate amounts of AA and UA stock solution to reach final concentrations of acids 10, 20, 30, 40, and 50 µg/mL. Mixtures were incubated at 37 °C. After 0, 2, 4, 6, and 24 h, samples were diluted and cultured on nutrient agar plates (BIOMED, Poland) for 18

h at 37 °C to determine the number of bacterial cells (CFU/mL). The control sample had no acids. The experiment was repeated 3 times.

2.6. Bacterial culture conditions used in experiments testing the impact of AA and UA on virulence factors

The bacteria were grown overnight at 37 °C in the presence of AA and UA (10, 20, 30, 40, and 50 μg/mL). Next, bacteria were harvested by centrifugation and resuspended in PBS to reach a final concentration of approximately 10^6 CFU/mL. Control samples had neither AA nor UA. The choice of these concentrations of triterpenes was due to the low bioavailability of both AA and UA for eukaryotic organisms (Jager et al., 2008).

2.7. Hemagglutination assay and expression of P-fimbriae

Equal volumes of bacterial suspension and 3% solution of human erythrocytes with or without D-mannose were mixed to determine the type of P-fimbrial mannose-resistant hemagglutination (Evans et al., 1980). The experiment was repeated 3 times.

2.8. Curli fibers expression

Ten microliters of bacterial suspension was inoculated onto a plate containing YESCA agar supplemented with congo red. Curli-producing *E. coli* bound the congo red dye and formed red colonies, whereas curli-negative bacteria formed white colonies (Hammar et al., 1995). The experiment was repeated 3 times.

2.9. Hemolytic activity

Ten microliters of bacterial suspension was spot-inoculated onto sheep-blood agar plates. Alpha-hemolysin production was confirmed by the appearance of a hemolysis zone around the spot (Ghenghesh et al., 2009). The experiment was repeated 3 times.

2.10. Swimming motility assay

Ten microliters of bacterial suspension was inoculated onto motility plates (1% tryptone, 0.25% NaCl, and 0.3% agar). After 24 h incubation at 37 °C, the diameters of the swimming zones were measured (Sanchez-Torrez et al., 2011). The presented results are the mean of 3 experiments.

2.11. Statistical analysis

The correlations between the concentrations of pentacyclic triterpenes and the presence of virulence factors (P-fimbriae, curli fibers, alpha-hemolysin) were analyzed by assessing the distribution of the variables using Pearson's test. The differences in growth and motility between rods exposed to AA and UA and those which were unexposed were analyzed by a t-test for independent samples. All tests were analyzed at the significance level $P < 0.05$ using Statistica 7.1.

3. Results and discussion

3.1. Molecular characterization of bacterial strain

Pyelonephritis is a potentially life-threatening infection that may lead to significant damage and kidney failure, abscess formation, and sepsis. The important role of *pap* and *hlyA* genes in the pathophysiology of pyelonephritis caused by *E. coli* has been reported in several studies (Moreno et al., 2005; Farshad and Emamghorashi, 2009). In the current study, we found that among the examined *E. coli* strains, the prevalence of virulence factors in genes ranged from 0% for *afa* and *draE* to 100% for *csgA*. The presence of the *papC* gene was detected in 17 (85%) strains. The *HlyA* gene encoding alpha-hemolysin was found in the genome of 12 *E. coli* rods (60%). All tested UPECs were divided into 4 pathotypes on the basis of the presence of virulence-related genes (Table 1). More than half of the tested rods belonged to pathotype 1.

Our results highlight a higher frequency of *csgA* and *papC* compared to the rest of the tested genes. This may indicate that the presence of these 2 genes is characteristic for the strains isolated from the upper parts of the urinary tract. The importance of P-fimbriae coded by the *pap* operon in pyelonephritis is well documented (Bogyiova et al., 2002; Johnson et al., 2005). However, there are no reports confirming that the *csgA* gene plays a significant role in the pathogenesis of pyelonephritis. The presence of curli fibers coded by this gene allows bacteria to bind to the host cell membrane proteins, colonize the renal tubular epithelium more efficiently, and produce biofilm. The prevalence of the *papC* gene among clinical isolates collected by us corresponds to results reported earlier (Borisova et al., 2004; Wojnicz et al., 2012). However, the distribution of the *hlyA* gene found in the current study was higher than reported by Arisoy et al. (2008) and

Table 1. Pathotype patterns of *E. coli* strains.

Pathotype (genotype)	Numbers (%) of all strains (n = 20)	Strain no.
Pathotype 1 (*papC*+, *csgA*+, *hlyA*+, *afa*-, *draE*-)	11 (55)	060, 11, 14, 192, 419, 448, 486, 529, 590, 679, 964
Pathotype 2 (*papC*+, *csgA*+, *hlyA*-, *afa*-, *draE*-)	6 (30)	30, 205, 399, 618, 652, 824
Pathotype 3 (*papC*-, *csgA*+, *hlyA*+, *afa*-, *draE*-)	1 (5)	794
Pathotype 4 (*papC*-, *csgA*+, *hlyA*-, *afa*-, *draE*-)	2 (10)	306, 338

+ present gene, - absent gene.

Farshad et al. (2009). Due to its strongly toxic effect and its ability to damage the membrane of renal cells, alpha-hemolysin may be an important factor in pyelonephritis (Tseng et al., 2002; Kaper et al., 2004). On the other hand, none of the tested strains possessed *afa* or *draE* genes. The frequency of these genes in bacteria causing pyelonephritis is very low (Matar et al., 2005; Santo et al., 2006). Such high prevalence of *csgA*, *papC*, and *hlyA* genes among rods tested in the current study indicates that the virulence traits encoded by these genes are essential for bacteria causing pyelonephritis. For this reason we decided to examine the antibacterial activities of AA and UA against UPECs and their effect on the synthesis of P-fimbriae and curli fibers, production of alpha-hemolysin, and swimming motility.

3.2. Antibacterial activity

In our study we used AA, the therapeutic activity of which is poorly described, and UA, the antimicrobial action of which, mainly against gram-positive bacteria, has been described in several reports (Chandramu et al., 2003; Cunha et al., 2007; Fontanay et al., 2008; Kurek et al., 2010). Table 2 summarizes the in vitro susceptibilities of the 20 isolates of *E. coli* to AA and UA. As previously identified (Wojnicz et al., 2012), MIC values were high and ranged from 512 μg/mL to >1024 μg/mL. Cunha et al. (2007) noted relatively low MIC values of UA against gram-positive bacteria. This triterpene displayed the most intense antibacterial effect against oral pathogens *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus sobrinus*, and *Enterococcus faecalis*, with MIC values ranging from 30 μg/mL to 80 μg/mL. UA was also active against *B. cereus*, showing the MIC value of 20 μg/mL (Cunha et al., 2010). Fontanay et al. (2008) noted very low MIC values of UA for reference strains *Staphylococcus aureus* ATCC 25923 (8 μg/mL), *S. aureus* ATCC 29213 (8 μg/mL), and *E. faecalis* ATCC 29212 (4 μg/mL). The MICs of UA were much higher (≥256 μg/mL) against gram-negative reference strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and clinical isolates of *P. aeruginosa*, penicillinase-producing *E. coli*, gram-positive methicillin-resistant *S. aureus*, and vancomycin-resistant enterococci. As we expected, the MIC values of UA were also very high, and for the majority of the tested strains were ≥1024 μg/mL. Only 10% of rods (2 strains) were sensitive to the UA at a concentration of 512 μg/mL. We also determined the MIC values of AA and found that the growth of 30% of UPECs (6 strains) was totally inhibited in the presence of 512 μg/mL of AA. Contrary to our results, Norzaharaini et al. (2011) evaluated the inhibitory effect of AA against both gram-negative (*Helicobacter pylori* ATCC 45903, *E. coli* ATCC 29952, *P. aeruginosa*) and gram-positive (*S aureus*, *S. pneumoniae*) strains at concentrations of 20 μg/mL. This AA concentration suppressed the growth of all the investigated bacteria except *P. aeruginosa*. Garo et al. (2007) noted that the MIC of AA against *P. aeruginosa* was 128 μg/mL. Djoukeng et al. (2005) showed that the MICs of AA fractions obtained from the extract of *Syzygium guineense* were much higher for gram-negative *Shigella sonnei* and *E. coli* strains in comparison with gram-positive *B. subtilis*. These values were 30.0, 5.0, and 0.75 μg/mL, respectively. Such differences in antigrowth activities of UA and AA against gram-positive and gram-negative bacteria may be caused by variations in the composition and permeability of the external surface structures of these microorganisms. The outer membrane of gram-negative bacteria may limit the activity of pentacyclic triterpenes (Horiuchi et al., 2007).

3.3. Effect of AA and UA on bacterial growth

The effects of AA and UA on the growth of *E. coli* strains are shown in Figure 2. These results show that bacterial

Table 2. The MICs of AA and UA against *E. coli* strains.

Strain no.	Concentrations of acids (μg/mL)		Strain no.	Concentrations of acids (μg/mL)	
	AA	UA		AA	UA
060	1024	1024	448	>1024	>1024
11	512	>1024	486	512	>1024
14	>1024	512	529	>1024	1024
30	>1024	>1024	590	1024	1024
192	512	1024	618	512	1024
205	>1024	1024	652	1024	1024
306	1024	>1024	679	1024	>1024
338	1024	512	794	1024	1024
399	512	>1024	824	1024	1024
419	512	1024	964	1024	>1024

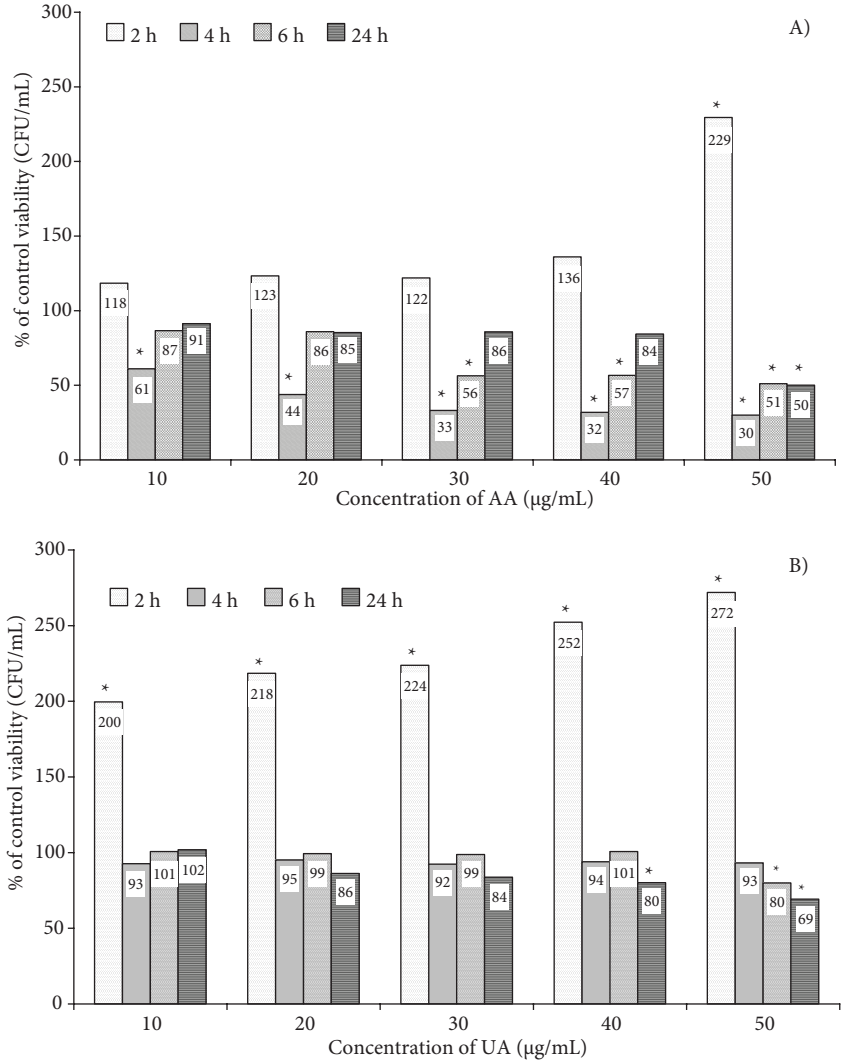

Figure 2. Effect of AA (A) and UA (B) on bacterial growth. Values represent the mean percentage of control viability (CFU/mL) for 20 *E. coli* strains. *: Result is statistically significant ($P < 0.05$).

growth depends on both the incubation time of UPECs in the presence of AA or UA and the concentration of triterpenes. The mean number of bacterial cells (CFU/mL) at time T_0 was 5.98×10^6 (100% survival of bacteria). Significantly increased survival of tested rods was observed after 2 h of exposure to all concentrations of UA and 50 μg/mL of AA ($P < 0.05$). A 4-h incubation of bacteria in the presence of triterpenes caused a decrease in their growth. However, the percentage of viable bacteria significantly decreased only in suspensions containing AA ($P < 0.05$). The survival of tested strains increased in 6-h bacterial cultures in comparison with 4-h cultures, except for bacteria treated with 50 μg/mL of UA. After 24 h of incubation, significant reduction of bacterial growth was observed only in samples containing AA at a concentration of 50 μg/mL and UA at concentrations of 40 and 50 μg/mL ($P < 0.05$).

The results obtained in this study show differences in growth-inhibitory activities between AA and UA (Figure 2). The survival of bacteria incubated in the presence of AA decreased significantly in comparison with the survival of rods exposed to UA. This phenomenon may be related to the chemical structure of the examined triterpenes. AA and UA have similar chemical structures (Figure 1), although it should be noted that the number of methyl and hydroxyl groups is different in these 2 acids. AA has 6 methyl groups attached to C4, C8, C10, C14, C19, and C20, while UA contains 1 more methyl group at the C4 position. AA possesses 3 hydroxyl groups attached to carbon atoms 2, 3, and 23 (C2, C3, C23) of the cyclic ring system; UA has only 1 hydroxyl group at C3. It is interesting that in the current study AA has shown more effective antigrowth activity than UA. Such an effect may

be related to the presence of the hydroxyl group at position C23 of AA (38). Research conducted by Wen et al. (2005) on the relationships between structure and activities of pentacyclic triterpenes showed that the A-ring structure also has a significant impact on their biological activities.

3.4. Hemagglutination assay and expression of P-fimbriae

Of the 17 *papC*+, *afa*-, and *draE*- strains, 15 (88%) caused agglutination of erythrocytes as a result of P-fimbriae expression. The effects of AA and UA on hemagglutination and expression of P-fimbriae are shown in Tables 3 and 4. The loss of hemagglutination abilities under the action of AA was observed for 6 *E. coli* strains and for 5 rods incubated in the presence of UA. The *E. coli* strains belonging to pathotype 1 (Nos. 060, 11, 529, 964) and pathotype 2 (No. 824) were susceptible to both acids. Even the lowest acid concentrations used (10 μg/mL) caused inhibition of P-fimbriae expression in tested *E. coli* rods. However, the most efficient inhibitory effect was observed for higher AA and UA concentrations (40 and 50 μg/mL). Very similar results were also obtained in our previous study (Wojnicz et al., 2012); however, we did not consider the genetic pathotypes in that study, but only analyzed the strains phenotypically. The analysis of the results presented in Tables 3 and 4 shows that the antifimbrial activities of these acids are relatively similar. However, statistical analysis of the results showed a significant correlation only between the concentrations of UA and the loss of P-fimbriae ($P < 0.05$). In the case of AA, the P-value was greater than 0.05, and therefore the null hypothesis ("variables dependent on each other") was rejected. This result means that the loss of P-fimbriae is independent of the AA concentrations.

3.5. Curli fiber expression

The impact of AA and UA on the occurrence of curli fibers is shown in Tables 3 and 4. The presence of the *csgA* gene, which encodes the structural protein curli fimbriae, was observed in all tested strains. Despite the presence of the *csgA* gene, 2 strains did not possess curli fibers and therefore formed white colonies on YESCA agar. Inhibition of curli fimbriae synthesis after exposure to AA was observed in 4 *E. coli* strains belonging to pathotype 1 (No. 11) and pathotype 2 (Nos. 30, 399, 618). Three *E. coli* isolates (Nos. 060, 11, 618) did not possess curli fibers after treatment with UA. The loss of congo red-binding abilities by the tested rods was observed only at concentrations of 40 and 50 μg/mL of both acids used. We obtained similar results in our previous research (Wojnicz et al., 2012). Results of the current study indicate that AA and UA have relatively similar inhibitory activities on curli fiber synthesis, although it should be noted that AA caused the

Table 3. The loss of virulence factors by *E. coli* strains exposed to AA.

Virulence factor	Concentrations of AA (μg/mL)									
	10		20		30		40		50	
	n	Strain no.	n	Strain no.	n	Strain no.	n	Strain no.	n	Strain no.
P-fimbriae (n = 15)	3	060, 11, 824	5	060, 11, 824, 529, 964	5	060, 11, 824, 529, 964	6	060, 11, 824, 529, 964, 590	6	060, 11, 824, 529, 964, 590
Curli fibers (n = 18)	0	---	0	---	0	---	3	11, 30, 399	4	11, 30, 399, 618
Alpha-hemolysin (n = 11)	0	---	0	---	1	192	2	192, 060	7	192, 060, 14, 419, 448, 679, 964

Table 4. The loss of virulence factors by *E. coli* strains exposed to UA.

Virulence factor	Concentrations of UA (μg/mL)									
	10		20		30		40		50	
	n	Strain no.	n	Strain no.	n	Strain no.	n	Strain no.	n	Strain no.
P-fimbriae (n = 15)	3	11, 824, 529	5	11, 824, 529, 060, 964	5	11, 824, 529, 060, 964	5	11, 824, 529, 060, 964	5	11, 824, 529, 060, 964
Curli fibers (n = 18)	0	---	0	---	0	---	1	11	3	060, 11, 618
Alpha-hemolysin (n = 11)	0	---	0	---	0	---	4	14, 419, 448, 679	8	14, 419, 448, 679, 060, 192, 590, 964

loss of curli fibers from cell surfaces of 7 strains, while UA caused loss in only 4 cases. Moreover, the statistical analysis of the data showed that the loss of curli fibers remained in correlation only with UA ($P < 0.05$). The loss of these adhesins by strains incubated in the presence of AA is independent of its concentration ($P > 0.05$).

3.6. Hemolytic activity

The effects of AA and UA on alpha-hemolysin production are shown in Tables 3 and 4. Of the *hlyA*+ strains, 11 out of 12 (73%) produced alpha-hemolysin. The inhibitory effect of the acids was observed in 8 *E. coli* strains belonging to pathotype 1 (Nos. 060, 14, 192, 419, 448, 590, 679, 964). The strongest antihemolytic activities were obtained during the treatment of tested bacterial strains with the highest (50 µg/mL) concentrations of both acids. The loss of alpha-hemolysin synthesis was also observed in 4 bacterial cultures exposed to UA, and in 2 bacterial suspensions containing AA, at a concentration of 40 µg/mL. At a concentration of 30 µg/mL, only AA inhibited the release of the toxin to the bacterial medium. Based on statistical analysis, we found that a correlation between concentration and the loss of hemolytic activity is present only in UA. No correlation was noticed for AA ($P > 0.05$), which means that the loss of the toxin synthesis can be observed in low as well as higher concentrations of this triterpene.

3.7. Swimming motility

The ability to move was observed in 14 (70%) out of 20 *E. coli* strains. The results are shown in Figure 3. The mean swimming zone diameter of bacteria in the control sample was 29.8 ± 2.0 mm. The diverse effect of both acids on swimming motility of UPEC strains was recorded. Bacterial movement of all strains was reduced at all concentrations of both triterpenes, but results were statistically significant only for the highest concentrations of AA and UA ($P < 0.05$). The mean zone diameters for bacteria treated with AA and UA at concentrations of 40 µg/mL were 23.1 ± 3.4 and 23.6 ± 3.4 mm, respectively. A slightly stronger inhibitory effect was noticed for bacteria incubated in the presence of both acids at a concentration of 50 µg/mL. The zone diameters were 22.2 ± 3.1 and 21.1 ± 2.9 mm for AA and UA, respectively.

The above results show that triterpenes do not differ significantly from each other in their inhibitory effects on bacterial virulence factors. Both AA and UA were most effective in reducing bacterial swimming motility and the loss of P-fimbriae. The movement of all strains was decreased at each concentration of both AA and UA, but statistically significant results occurred at only the 2 highest concentrations ($P < 0.05$). Strong anti–P-fimbrial effect was also observed in all concentrations of both triterpenes. The loss of curli fibers and alpha-hemolysin synthesis was correlated only with the highest concentrations of UA ($P < 0.05$). No statistical significance between AA concentration and the suppression of these virulence factors was found ($P > 0.05$).

In this study, a lower level of antigrowth activity was shown for UA than AA. This result may be due to a smaller number of hydroxyl groups in the A-ring of UA in comparison with AA. UA possesses only one hydroxyl group and therefore has a more hydrophobic character than AA. Hence, the penetration of UA across the outer membrane of *E. coli* strains is hindered. Raetz and Whitfield (2002) established that lipid A (a component of lipopolysaccharide) anchored in the outer membrane

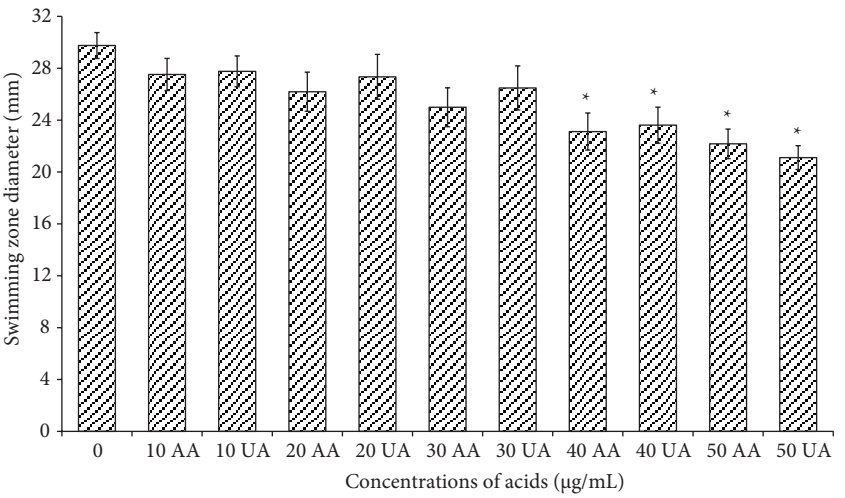

Figure 3. Effect of AA and UA on swimming motility. Values represent the mean value of swimming zone diameter (mm) for 14 *E. coli* strains. Bars indicate standard deviations. *:Result is statistically significant ($P < 0.05$).

is a barrier inhibiting the penetration of hydrophobic substances. AA has a more hydrophilic character because of the 3 hydroxyl groups in its structure and therefore can more easily penetrate the outer membrane, thus demonstrating higher antibacterial activity.

In conclusion, pentacyclic triterpenes may serve as supplementary agents that could improve standard conventional antibacterial therapy in UTIs. However, the action of pentacyclic triterpenes on bacterial cells is still not fully known and requires further investigation.

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