ANTIMICROBIAL ACTION OF A-ARBUTIN, B-ARBUTIN AND HYDROQUINONE: TRUTH AND FICTION

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Introduction. Arbutin (C12H16O7) is a glucopyranoside of hydroquinone with two different configurations: alpha (α) and beta (β). The β-isomer can only be obtained from medicinal raw materials, and in turn, the α-isomer is its synthetic analog; the main difference between these isomers is that instead of β-glucose there is α-glucose. Hydroquinone is an aromatic compound which consists of benzyl and 2 OH groups in para-positions. Hydroquinone is a highly toxic compound and is carcinogenic. [1]. (Fig. 1)

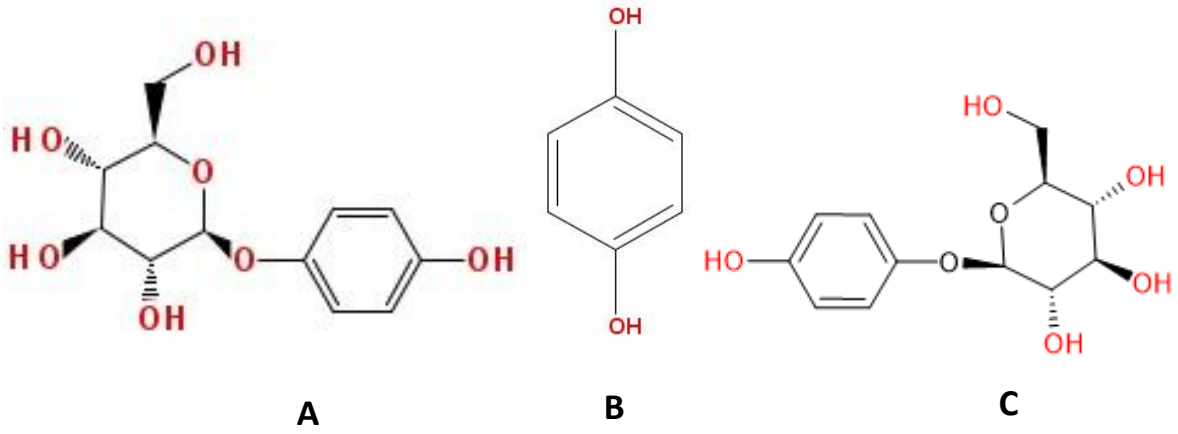

Figure 1. Structural formula of β-arbutin (A), hydroquinone (B) and α-arbutin (C)

β-Arbutin is found in the leaves of medicinal plants of the *Ericaceae* and *Saxifragaceae* families, arbutin was first isolated in 1852 from the leaves of the common strawberry tree (*Arbutus unedo* L.) of the *Ericaceae* family. The main natural sources of β-arbutin are the leaves of bergenia crassifolia (arbutin up to 20% in raw materials), in second place are the leaves of bearberry (arbutin 5-12% in raw materials), and in third place are the leaves of lingonberry (arbutin up to 5-8% in raw materials). [2].

Unlike β-arbutin, α-arbutin is obtained by chemical synthesis of hydroquinone and glucose using the Koenig-Knorr glycosylation method, but this method is quite multi-stage due to the need to protect and remove protection from the hydroxyl groups of hydroquinone. Thus, the biosynthetic approach to obtaining α-arbutin is rapidly gaining momentum, since the protection/removal of protection steps are not necessary. [3].

α- and β-arbutin have anti-inflammatory [4], antioxidant [5], and anti-cancer effects [6]. Arbutin is also used in cosmetology for skin lightening, since arbutin is a highly selective tyrosinase inhibitor [7]. A number of studies have shown that the pharmacological action of the α-isomer is significantly higher than that of the β-isomer, for example, α-arbutin is 10 times more active in inhibiting tyrosinase than the β-isomer [8], which indicates the importance of conducting a comparative analysis of other pharmacological activities of arbutin isomers.

All over the world, arbutin-containing medicinal raw materials (lingonberry and bearberry leaves) are used for the treatment and prevention of cystitis, glomerulonephritis and pyelonephritis, as uroseptic, diuretic and antiazotemic agents [9]. In the international community [10], there is a theory that the mechanism of the diuretic and antimicrobial action of arbutin is that arbutin, under the influence of hydrochloric acid in the stomach, is hydrolyzed to hydroquinone, which irritates the renal tissue and increases urination, and hydroquinone inhibits the growth of bacteria in the urinary tract.

In our opinion, this justification contains a number of contradictions: firstly, hydroquinone is a highly toxic compound, the lethal dose is from 50 to 500 mg/kg or from 200 to 2000 mg per 70 kg (oral administration) [11], hydroquinone inevitably affects the kidneys, liver and nervous system, further, according to the official publication of Mashkovsky M.D. "Medicines" 16th ed. [12], bearberry leaves are used as a diuretic and antiazotemic agent in the form of a decoction as follows: "*Pour 10 g into 200 ml of hot boiled water, heat in boiling water for 30 minutes, cool for 10 minutes at room temperature, filter. Squeeze out the residue, bring the infusion volume to 200 ml with boiled water. Take 1/3 - 1/2 cup of decoction 3-4 times a day 40 minutes after meals*", with a conditional calculation ($arbutin_{mg/day}$ = (10 g × 6%) / 100 = 0.6 g) the patient consumes up to 600 mg of arbutin /hydroquinone per day every day, but this dose is lethal, while in medical practice we do not observe the toxic manifestation of arbutin after taking a decoction of bearberry and lingonberry leaves; secondly, according to the above theory, arbutin has a uroseptic effect only due to the action of hydroquinone and nothing more, it turns out that

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arbutin does not have an antimicrobial effect at all, which is also a contradiction. In our opinion, the discussion of the theories of the mechanisms of uroseptic and diuretic action of arbutin is still open in the scientific community and requires careful study not only from the side of pharmacology and pharmacognosy, but also toxicological chemistry.

Thus, the aim of our study was to investigate the *in vitro* and *in silico* antimicrobial action of α- and β-arbutin, hydroquinone, and also to conduct a comparative analysis of the antimicrobial properties of these compounds and to refute the theory of the presence of the antimicrobial action of arbutin only due to the action of hydroquinone.

Materials and methods

β-Arbutin (≥98.0%) was purchased in Sigma Aldrich Company, Lublin, Poland; α-arbutin (≥98.0%) and hydroquinone were provided by pharmaceutical company "Zdravopharm", Kharkiv, Ukraine.

The method of diffusion of the drug into agar carried out using the method of "wells" [13, 14]. Table 1 shows interpretation criteria for microbial sensitivity.

Table 1. **Interpretation criteria for microbial sensitivity**

Microbial sensitivity	Diameter of the growth retardation zone, mm
High sensitivity	>25
Sensitive	15-25
Low sensitivity	10-15
Not sensitivity	<10

Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Bacillus subtilis ATCC 6538, Candida albicans ATCC 885/653, Proteus vulgaris NTCS 4636 и *Pseudomonas aeruginosa ATCC 27853* were used in accordance with established guidelines for assessing the antimicrobial efficacy of pharmaceuticals.

The MIC is defined as the lowest concentration of an antibacterial agent that completely prevents bacterial growth. The MIC for various extracts was determined using the broth microdilution method [15]. The concentration of β- and α-arbutin, hydroquinone was 0.2 mol/L.

The molecular docking study was performed using a tool known as AutoDockTools 1.5.6 [16]. Protein preparation involved an optimization process that involved removing water and other atoms, followed by adding a polar hydrogen group. Autogrid was used to set up the grid coordinates (X, Y, and Z) at the binding site. Genetic algorithm parameters were applied to the ligand interaction with 10 runs of this criterion. The theoretical study of antimicrobial activity against Gram-negative strains was conducted against the following *P. aeruginosa* enzyme structures: DNA gyrase (PDB ID: 1KIJ), DHFR (PDB ID: 1RX3), deacetylases (PDB ID: 3UHM), while against Gram-positive strains, the following *S. aureus* enzymes were studied: DNA gyrase (PDB ID: 1KZN), DHFR (PDB ID: 3FRA), penicillin-binding protein 3 (PDB ID: 3UHM), while against the fungal strain the following *C. albicans* enzymes were studied: beta-1,3-glucanase (PDB ID: 1EQP), thymidylate kinase (PDB ID: 5UIV), squalene epoxidase (PDB ID: 6C6R), 14alpha-demethylase (PDB ID: 6AYB). All structures were obtained from the PDB database [12]. The resolution of 1KIJ was 2.30 Å, 1RX3 – 2.20 Å, 3UHM – 2.20 Å, 1KZN – 2.30 Å, 3FRA – 2.35 Å, 3UHM – 2.26 Å, 1EQP – 1.87, 5UIV – 2.45, 6C6R – 3.00, 6AYB – 1.87. The protein structure is selected for the docking experiment if the resolution is higher than 1 Å. Thus, all mentioned proteins can be used for the experiment. Structures of ligands of β-arbutin (CID_440936), hydroquinone (CID_785), α-arbutin (CID_158637); gentamicin (CID_3467); fluconazole (CID_3365) were obtained from the PubChem database [13]. The active site of the docking protein was identified using the Computed Atlas for Surface Topography of Proteins (CASTp) [14].

Data in the tables are presented as $X \pm SD$ (mean ± standard deviation). Differences were considered significant at $P < 0.05$.

Results and discussion

A theoretical study of the antibacterial and antifungal activities of α-arbutin, β-arbutin and hydroquinone was conducted using molecular docking to understand their promising capabilities in inhibiting the growth of gram-negative, gram-positive bacterial and fungal strains. The antimicrobial effect was evaluated with 3 key enzymes of the "first line of defense" for gram-negative strains: DNA gyrase, DHFR, deacetylase; and gram-positive strains: DNA gyrase, DHFR, penicillin-binding protein 3; and 4 essential enzymes of fungi: beta-1,3-glucanase, thymidylate kinase, squalene epoxidase, 14alpha-demethylase.

There are many works on molecular docking in the study of the pharmacological activity of various groups of compounds in the indexed scientific journals Scopus and Web of Science. However, the main problem of these studies is the lack of a rating assessment of the efficiency of ligand binding to the active center. Several scientific papers used comparison standards; however, in our opinion, this method is not promising since more than one standard can be used for the studied protein enzyme. Thus, such an assessment method will lead to confusion in the data among scientists. To understand the level of selectivity of inhibition of the active centers of bacterial enzymes by the studied substances, we used the following classification of selectivity [15]: IC50 < 0.001 mM (highly selective); 0.05 > IC50 > 0.01 (medium selectivity); IC50 > 0.05 mM (low selectivity) [20].

Molecular modeling of the identified compounds was performed with the active site of DNA gyrase. The active site was represented by the following amino acids: Arg75, Lys102, Arg135, Asp80. Trp387, Lys109, Asp72 and Thr166. β-arbutin showed high selectivity to the enzyme's active site, while α-arbutin had medium selectivity, and hydroquinone had low selectivity. Antibacterial standards such as chloramphenicol were medium selective inhibitors, and gentamicin was a low selective inhibitor. The binding

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free energy of β-arbutin was 5 and 54% higher than that of α-arbutin and hydroquinone, respectively. (Table 2)

Table 2. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antimicrobial drug standard with the DNA-gyrase structure of *P. aeruginosa*

№	Ligand	DNA-gyrase		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	β-Arbutin	-8.23	0.00093344	High selective
2.	α-Arbutin	-7.80	0.00192	Medium selective
3.	**Chloramphenicol**	-6.38	0.02114	Medium selective
4.	**Gentamycin**	-4.08	1.03	Low selective
5.	Hydroquinone	-3.73	1.86	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The next enzyme studied was DHFR. The active center of this enzyme was represented by the following amino acids: NADP, Tyr110, Asp30, Ile8, Phe34, Ile104, Arg55, Arg60. According to the results presented in Table 3, the free energy of binding decreased in the following order: β-arbutin (-9.17) < α-arbutin (-9.00) < chloramphenicol (-7.97) < gentamicin (-6.78) < hydroquinone (-4.56). Highly selective inhibitor was α- and β-arbutin, while hydroquinone had low selectivity. β-arbutin had higher affinity of 2 and 50% than α-arbutin and hydroquinone. (Table 3)

Table 3. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antimicrobial drug standards with the DHFR structure of *P. aeruginosa*

№	Ligand	DHFR		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	β-Arbutin	-9.17	0.00019	High selective
2.	α-Arbutin	-9.00	0.000188	High selective
3.	**Chloramphenicol**	-7.97	0.00143	Medium selective
4.	**Gentamycin**	-6.78	0.01073	Medium selective
5.	Hydroquinone	-4.56	0.45322	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Molecular modeling of the studied compounds was carried out with the active center of Deacetylase. The active center was represented by the following amino acids: Thr190, Lys238, Gly92. Phe191, Leu18, Ala206. Table 4 demonstrates that β-arbutin has high selectivity, α-arbutin has medium selectivity, while hydroquinone has the lowest level of selectivity to the active center. While antibacterial standards gentamicin and chloramphenicol have medium selectivity. The free binding energy of β-arbutin was 7 and 47% stronger compared to α-arbutin and hydroquinone.

Table 4. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antimicrobial drug standard with the deacytelese structure of *P. aeruginosa*

№	Ligand	Deacetylase		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	β-Arbutin	-8.40	0.00070	High selective
2.	α-Arbutin	-7.80	0.00192	Medium selective
3.	**Gentamycin**	**-7.45**	0.00346	Medium selective
4.	**Chloramphenicol**	**-7.19**	0.00536	Medium selective
5.	Hydroquinone	-4.46	0.54165	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The study of the theoretical potential of the antimicrobial action of the studied compounds against gram-positive strains was initiated with respect to the DNA-gyrase structure of S. aureus. The active center was represented by the following amino acids: Val43, Asn46, Val71, Pro79, Ile90, Asp73, Arg76, Gly77, Arg136. According to the results of the study and conditional assessment, it was established that all compounds have low selectivity for the active center of the DNA-gyrase structure. The affinity of β-arbutin was 5 and 43% higher than that of α-arbutin and hydroquinone, respectively. (Table 5)

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Table 5. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antimicrobial drug standard with the DNA-gyrase structure of *S. aureus*

№	Ligand	DNA-gyrase		
		ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1.	β-Arbutin	-5.48	0.09629	Low selective
2.	α-Arbutin	-5.20	0.09400	Low selective
3.	**Chloramphenicol**	**-4.30**	0.7071	Low selective
4.	Hydroquinone	-3,12	5.18	Low selective
5.	**Gentamycin**	**-1.23**	126.20	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The next enzyme studied was DHFR. The active center of this enzyme was represented by the following amino acids: Ala7, Ile14, Asn18, Gln19, Gly43, Arg44, Lys45, Thr46, Thr63, Ser64, His77, Gly94, Gln95, Thr96, Leu97, Tyr98, Glu100. According to the results presented in Table 6, none of the compounds had high selectivity. The affinity for the active center of the DHFR structure decreased in the following order: chloramphenicol > gentamicin > β-arbutin > α-arbutin > hydroquinone. The free energy of binding of β-arbutin was 2 and 48% higher than that of α-arbutin and hydroquinone.

Table 6. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antimicrobial drug standard with the DHFR structure of *S. aureus*

№	Ligand	DHFR		
		ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1.	**Chloramphenicol**	**-7.29**	0.00454	Medium selective
2.	**Gentamycin**	**-7.15**	0.00575	Medium selective
3.	β-Arbutin	-6.19	0.02903	Medium selective
4.	α-Arbutin	-6.18	0.02900	Medium selective
5.	Hydroquinone	-3.56	2.47	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Molecular modeling of the studied compounds in relation to gram-positive strains was carried out using the structure of the active center of the penicillin-binding protein. The active center was represented by the following amino acids: Ala622, Glu623, Asn633, Pro660. According to the results of the study and conditional classification, it was found that neither antibiotics nor natural compounds (arbutin, hydroquinone) have high selectivity. The affinity of β-arbutin to the active center was 5, 1, and 40% stronger than that of α-arbutin, fluconazole, and hydroquinone. (Table 7)

Table 7. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antimicrobial drug standard with the penicillin-binding protein structure of *S. aureus*

№	Ligand	Penicillin-binding protein		
		ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1.	β-Arbutin	-4.47	0.53	Low selective
2.	α-Arbutin	-4.25	0.45	Low selective
3.	**Chloramphenicol**	**-4.06**	1.05	Low selective
4.	Hydroquinone	-3.56	2.47	Low selective
5.	**Gentamycin**	**-3.41**	3.19	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The theoretical antifungal study was started with beta-1,3-glucanase enzyme. The active center was represented by the following amino acids: Leu304, Tyr29, Tyr255, Trp363, Glu292, Asn146, Glu27. Table 8 showed that β-arbutin and α-arbutin were highly selective inhibitors, while hydroquinone was a low-selective inhibitor. Compared with the antifungal standard fluconazole, the affinity of β-arbutin was 5.0, 16.0, and 50.0% compared with α-arbutin, fluconazole, and hydroquinone.

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Table 8. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antifungal drug standard with the beta-1,3-glucanase structure of *C. albicans*

№	Ligand	beta-1,3-glucanase		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	β-Arbutin	-9.17	0.000189	High selective
2.	α-Arbutin	-9.00	0.000170	High selective
3.	**Fluconazole**	-7.71	0.00554	Medium selective
4.	Hydroquinone	-4.61	0.42	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The next enzyme evaluated by molecular docking was thymidylate kinase. According to the results, β-arbutin, fluconazole, and α-arbutin were highly selective inhibitors, while hydroquinone had the lowest selectivity. The binding energy of β-arbutin was 1, 4, and 56% higher than that of fluconazole, α-arbutin, and hydroquinone. (Table 9)

Table 9. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antifungal drug standard with the thymidylate kinase structure of *C. albicans*

№	Ligand	Thymidylate kinase		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	β-Arbutin	-9.13	0.000204	High selective
2.	**Fluconazole**	**-9.12**	0.000207	High selective
3.	α-Arbutin	-8.99	0.000170	High selective
4.	Hydroquinone	-3.99	1.19	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Molecular modeling of the studied compounds was carried out with the active site of squalene epoxidase. The active center was represented by the following amino acids: Tyr532, Ile528, Leu497, Cys501, Tyr494. Flucinazole had the medium selectivity, whereas β-arbutin, hydroquinone had the lowest selectivity. (Table 10)

Table 10. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antifungal drug standard with the squalene epoxidase structure of *C. albicans*

№	Ligand	squalene epoxidase		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	**Fluconazole**	**-7.06**	0.00665	Medium selective
2.	β-Arbutin	-5.80	0.0565	Low selective
3.	α-Arbutin	-5.70	0.0555	Low selective
4.	Hydroquinone	-3.49	2.74	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Molecular modeling of the studied compounds was carried out with the active site of 14alpha-demethylase. The active site was represented with amino acids: Thr297, Ala293, Hem501, Ala289, Phe114, Tyr120. The β-arbutin, α-arbutin and fluconazole had high selectivity to active site. β-arbutin affinity was 3, 10 and 60% higher than α-arbutin, fluconazole and hydroquinone, respectively. (Table 11)

Table 11. Results of molecular docking of the α-arbutin, β-arbutin, hydroquinone and antifungal drug standard with the 14alpha-demethylase structure of *C. albicans*

№	Ligand	14alpha-demethylase		
		ΔGbind[a] (kcal/mol)	**Ki[b] (mmol)**	**Level of selectivity**
1.	β-Arbutin	-9.45	0.000118	High selective
2.	α-Arbutin	-9.30	0.000110	High selective
3.	**Fluconazole**	**-8.53**	0.00056	High selective
4.	Hydroquinone	-5,02	0.211	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

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Further, antibacterial and anti-fungi drugs and arbutin, hydroquinone were conditionally divided into two categories. The first category included compounds that had a high selectivity for the active site, and the second category included compounds that had medium and low selectivity. This compound separation approach was necessary to clearly identify compounds that interact highly effectively with antibacterial and anti-fungi mechanisms and which compounds work below this level. According to the results shown in Table 12, there was only two compounds that inhibited all mechanisms against Gram-negative strains – α-arbutin and β-arbutin, whereas in the case of Gram-positive strains none of compounds were high selective. In the case of anti-fungi enzymes fluconazole actively inhibited 2 mechanisms out 4, whereas α- and β-arbutin inhibited 3 mechanisms and hydroquinone was not high selectively inhibited all 4 fungi mechanisms. (Table 12, 13)

Table 12. Schematic division of antibacterial standards and α-arbutin, β-arbutin, hydroquinone in two categories

№	Compound	Gram-negative strains (*P. aeruginosa, E. coli, P. vulgaris*)			Gram-positive strains (*S. aureus, B. subtilis*)			Gram-negative strains	Gram-positive strains
		DNA-gyrase	DHFR	Deacetylese	DNA-gyrase	DHFR	penicillin-binding protein	№ of inhibition enzymes of "First line of protection"	№ of inhibition enzymes of "First line of protection
Antibacteril drug standards									
1	**Chloramphenicol**							0/3	0/3
2	**Gentamycin**							0/3	0/3
Biological active compounds									
3	β-Arbutin							3/3	0/3
4	α-Arbutin							3/3	0/3
5	Hydroquinone							0/3	0/3

Table 13. Schematic division of anti-fungi standards and α-arbutin, β-arbutin, hydroquinone in two categories

№	Compound	Fungi strains (*C. albicans*)				*C. albicans*
		beta-1,3-glucanase	thymidylate kinase	squalene epoxidase	14alpha-demethylase	№ of inhibition enzymes
Anti-fungi drug standard						
1	**Fluconazole**					2/4
Biological active compounds						
2	β-Arbutin					3/4
3	α-Arbutin					3/4
4	Hydroquinone					0/4

β-arbutin had a significant inhibitory effect on colony growth against *S. aureus* (20.0 mm), *B. subtilis* (21.0 mm), *E. coli* (17.0 mm) and *C. albicans* (18.0 mm). α-arbutin had a stronger antibacterial effect on *S. aureus* (21.0 mm), *E. coli* (21.0 mm), *P. vulgaris* (20.0 mm) and *P. aeruginosa* (21.0 mm) than β-arbutin. While β-arbutin had a stronger antifungal effect on *C. albicans*. With respect to *S. aureus* (23.0 mm), *B. subtilis* (23.0 mm), *E. coli* (21.0 mm), *P. vulgaris* (16.00 mm) and *P. aeruginosa* (18.0 mm), the inhibitory effect of hydroquinone was higher than that of β-arbutin. At the same time, β-arbutin demonstrated a significantly stronger antifungal effect with respect to the fungal strain *C. albicans* (18.0 mm). α-arbutin had the greatest antimicrobial effect than hydroquinone on *P. vulgaris* (20.0 mm), *P. aeruginosa* (21.0 mm).

The antibacterial effect of gentamicin was 9.4% stronger against *S. aureus* than β- and α-arbutin, in the case of E. coli, the effect of gentamicin was 33.0, 17.0 and 17.0% higher than β- and α-arbutin, hydroquinone, respectively. On *P. vulgaris*, the inhibitory effect of gentamicin was 44.0, 20.0 and 36.0% stronger than β-

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and α-arbutin, hydroquinone, respectively. The antibacterial effect of gentamicin against *P. aeruginosa* was 38.0, 18.0 and 30.0% higher than β- and α-arbutin, hydroquinone, respectively. Fluconazole demonstrated a greater inhibitory effect by 10.0, 15.0 and 25.0% than β- and α-arbutin, hydroquinone, respectively. At the same time, chloramphenicol demonstrated a lesser inhibitory effect than β- and α-arbutin, hydroquinone against *S. aureus* (19.0 mm), *B. subtilis* (19.0 mm), *E. coli* (16.0 mm).

Table 14. Inhibition diameter (mm) resulting from the screening of antimicrobial effect against strains of *S. aureus, B. subtilis,P. aeruginosa, E. coli, P. vulgaris* and *C. albicans* by well diffusion method with α-arbutin, β-arbutin, hydroquinone and standards

Sample	Concentration, mmol/L	Diameter of the growth retardation zone					
		Gramm-positive strains		*Gramm-negative strains*			*Fungi strains*
		S. aureus ATCC 25923	*B. subtilis* ATCC 6538	*E. coli* ATCC 25922	*P. vulgaris* ATCC 4636	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 653/885
β-Arbutin	0.003	20.0±0.4	21.0±0.4	17.0±0.5	14.0±0.6	16.0±0.5	18.0±0.4
α-Arbutin	0.003	21.0±0.4	21.0±0.4	21.0±0.4	20.0±0.4	21.0 ±0.4	17.0±0.5
Hydroquinone	0.003	23.0±0.3	23.0±0.3	21.0±0.4	16.0±0.5	18.0±0.4	15.0±0.5
Gentamycin	0.003	22.0±0.2	20.0±0.4	25.3±0.3	25.0±0.2	25.7±0.2	12.0±0.7
Chloramphenicol	0.003	19.0±0.4	19.0±0.4	16.0±0.6	15.0±0.6	16.0±0.6	17.0±0.5
Fluconazole	0.003	—	—	—	—	—	20.0±0.4

The tested compounds β and α-arbutin significantly inhibited Gram-positive, Gram-negative and fungal strains with MIC. β-arbutin with MIC value of 0.00078 mM (1:256) was most active against S. aureus, B. subtilis. In case of α-arbutin, the highest MIC value was 0.00078 mM (1:256) against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. While hydroquinone had lower MIC values than α– and β-arbutin, hydroquinone was most active with MIC value of 0.00313 mM (1:64) against *S. aureus* and *B. subtilis*. For *P. aeruginosa*, *E. coli* and *C. albicans* the MIC values of the compounds decreased in the following order: hydroquinone (0.00625 mM (1:32)) > β-arbutin (0.00156 mM (1:128)) > α-arbutin (0.00078 mM (1:256)); in the case of P. vulgaris: hydroquinone (0.00125 mM (1:16)) > β-arbutin (0.00313 mM (1:64)) > α-arbutin (0.00156 mM (1:128)); for *S. aureus* and *B. subtilis* it was: hydroquinone (0.00313 mM (1:64)) > β- and α-arbutin (0.00078 mM (1:256)). (Table 15)

Table 15. Minimal inhibitory concentration of the α-arbutin, β-arbutin, hydroquinone against the strains of *S. aureus, B. subtilis, P. aeruginosa, E. coli, P. vulgaris* and *C. albicans*

Sample	MBIC, mM					
	Gramm-positive strains		*Gram0m-negative strains*			*Fungi strains*
	S. aureus ATCC 25923"	*B. subtilis* ATCC 6538	*E. coli* ATCC 25922	*P. vulgaris* ATCC 4636	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 653/885
β-Arbutin	0.00078 (1:256)	0.00078 (1:256)	0.00156 (1:128)	0.00313 (1:64)	0.00156 (1:128)	0.00156 (1:128)
α-Arbutin	0.00078 (1:256)	0.00078 (1:256)	0.00078 (1:256)	0.00156 (1:128)	0.00078 (1:256)	0.00156 (1:128)
Hydroquinone	0.00313 (1:64)	0.00313 (1:64)	0.00625 (1:32)	0.00125 (1:16)	0.00625 (1:32)	0.00625 (1:32)

In order to suppress the growth of any bacterium, it is necessary to effectively influence 3 main mechanisms: DNA gyrase, DHFR and inhibition of membrane formation. DNA gyrase is an enzyme responsible for the temporary division of bacterial DNA into two strands, which subsequently initiates the replication stage [21]. The next most important enzyme is DHFR; this enzyme is responsible for the formation of folic acid, which is necessary for the existence of bacteria [22, 23]. One of the main defense mechanisms of any bacterium is its membrane, and gram-negative strains are no exception to the rule. The membrane of gram-negative bacteria contains a special lipopolysaccharide that causes an immune system reaction and fever. The enzyme UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase is responsible for the synthesis of lipopolysaccharide; this enzyme has no homologues in

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humans and mammals and is present only in bacteria [24]. While the cell membrane of gram-positive bacteria contains peptidoglycan, this compound is a heteropolymer of polysaccharide and peptides that plays a key role in the construction of the cell membrane. Cross-linking of individual peptidoglycan peptides requires penicillin-binding proteins, which are the main target of lactams [25].

The cell membrane is the "first line" of defense not only for bacteria, but also for fungi. The key role in creating this defense is played by the compound ergosterol, which is necessary for constructing the cell membrane of fungi. To disrupt the biosynthesis of ergosterol, the following important enzymes should be inhibited: 14α-demethylase (catalyzes the reaction of converting lanosterol into ergosterol) and squalene epoxidase (catalyzes the stereoisomerization of squalene into 2,3(S)-oxidosqualene) [26]. The second component of the first "line of defense" of fungi is the polysaccharide β-1,3-glucan, this polysaccharide makes up to 55% of all membrane polysaccharides, it plays the role of a "framework" for the cell wall of fungi. For its synthesis, the enzyme β-1,3-glucan synthetase is required, which is responsible for the hydrolysis of the β-glucan chain by successive cleavage of residues from the unreduced end [27]. The next important component of any cell is its DNA, which is important for cell division. In this case, we assessed the ability of the studied substances to inhibit thymidylate kinase, the enzyme is responsible for the synthesis of pyrimidinated bases, when it is inhibited, a large number of "errors" occur in the DNA of fungi, leading to "inhibition" of the cell division process [28].

According to the obtained data, it was found that none of the compounds are highly selective inhibitors of all the mechanisms of action of bacteria and fungi, therefore, the creation of a "panacea" with antibacterial and antifungal action from one compound is impossible. β- and α-arbutin inhibited highly selectively the mechanisms of gram-negative bacteria, and in the case of fungi - three mechanisms out of four, while against gram-positive mechanisms β- and α-arbutin showed low selectivity. Meanwhile, hydroquinone turned out to be "useless" for all the presented mechanisms of bacteria and fungi, hydroquinone occupied the last places in the "strength" of affinity to the active center of the enzyme. Comparing the "natural" compounds with standards such as chloramphenicol, gentamicin and fluconazole, it was found that α- and β-arbutin showed higher results in selectivity for antibacterial and antifungal "targets". Gentamicin and chloramphenicol did not show high selectivity for gram-negative "targets", but the same trend was observed for gram-positive mechanisms of action. Meanwhile, fluconazole actively inhibits two "targets" out of four, which indicates its high ability to inhibit the growth of bacteria and fungi. Also, theoretical studies have shown that the difference in selectivity between α- and β-arbutin is no higher than 5%, which indicates the absence of a significant difference in their selectivity for "targets", but in practice their inhibitory effect may be different depending on the type of strain. Moreover, the binding energy of hydroquinone compared to α- and β-arbutin was almost 2-3 times less.

The next important stage of our study was to conduct an experimental study of the compounds under study. To assess the antimicrobial effect, we used the "well" method and the "dilution" method. The concentration of the compounds under study was presented in molar concentrations, and not in the generally accepted measure of "mg/mL" or "%", this is due to the fact that the mole reflects the amount of a substance (molecules) that will interact in a particular reaction. In the case of using "%", all the compounds under study will have a different number of molecules, since each has its own individual molecular mass and thus such a comparison of pharmacological activity is "incorrect" since one compound will have more molecules, and another will have less [29].

In the studies of antibacterial and antifungal activity of the compounds by the "well" method, it was shown that hydroquinone inhibits gram-positive bacteria more actively than β- and α-arbutin, and in the case of gram-negative bacteria, α-arbutin had a higher inhibitory effect than β-arbutin and hydroquinone, while β-arbutin actively inhibits the growth of fungi than α-arbutin and hydroquinone. Next, we assessed the antibacterial and antifungal activity of the studied compounds by the "dilution" method. As a result, it was found that the MIC of hydroquinone for gram-positive, gram-negative and fungi was almost 2 to 3 times greater than the MIC value of β- and α-arbutin. Meanwhile, the MIC of α-arbutin was lower for *E. coli*, *P. vulgaris* than β-arbutin, and in other cases the results were the same. Taking into account only the antimicrobial results obtained by the "wells" method, it may seem that hydroquinone is a stronger inhibitor than its glycoside forms. But, the "dilution" method showed that hydroquinone is significantly inferior to β- and α-arbutin, and in order to suppress the growth of bacteria and fungi, hydroquinone will need much more than its glycoside forms. When comparing theoretical and practical results, we can firmly say that the results are comparable in the case of the "dilution" method, and not in the case of the "wells" method. But, in our opinion, to understand the "strength" of the antimicrobial effect, it is necessary to carry out studies both by the "wells" method and by the "dilution" method.

Returning to the topic of "theories of uroseptic and diuretic action of arbutin" a number of important questions arise. According to the data presented above, hydroquinone inhibits the growth of bacterial and fungal colonies 2-3 times weaker than β- and α-arbutin. Thus, the "theory" that arbutin has uroseptic action only due to the action of hydroquinone is "refuted". Consequently, the "theory" developed and accepted by the "luminaries" in the 1930s requires revision and updating. Our hypothesis on the rationale for arbutin metabolism is as follows: "arbutin, when it enters the stomach, is not hydrolyzed to hydroquinone, after which arbutin is absorbed in the small intestine, part of arbutin enters the liver through the bloodstream, where it is metabolized to the glucorone form, and the other part enters the large intestine and is hydrolyzed to hydroquinone, and then is

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absorbed into the bloodstream and the liver, then in the glucorone form it enters the renal system, where it has a uroseptic and diuretic effect." This problem requires close attention and research from toxicologists, biochemists, microbiologists, and doctors.

Conclusions

The antimicrobial action of α- and β-arbutin, hydroquinone against strains of *S. aureus, B. subtilis, E. coli, P. vulgaris, P. aeruginosa* and *C. albicans* was studied *in vitro* and *in silico*. The theoretical results showed that it is impossible to create a "panacea" from one compound that inhibits the growth of both bacteria and fungi. According to theoretical and practical results, the antimicrobial action of α- and β-arbutin is 2-3 times higher than that of hydroquinone. It was experimentally confirmed that α-arbutin inhibits the growth of gram-negative strains much more strongly than β-arbutin. The theory that arbutin has an antimicrobial effect only due to the action of hydroquinone was refuted theoretically and practically.

Antimicrobial action of α-arbutin, β-arbutin and hydroquinone: truth and fiction

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Introduction. The leaves of lingonberry and bearberry are used in medicine for the treatment and prevention of urological infectious diseases due to the presence of diuretic and uroseptic action. This pharmacological activity is associated with the action of β-arbutin and hydroquinone. However, until now there has been no study of the relationship between the structure and antimicrobial action of β-arbutin and hydroquinone. **The purpose of study was** to estimate the antimicrobial action *in vitro*, *in silico* of α- and β-arbutin, hydroquinone, and also to conduct a comparative analysis of the antimicrobial properties of these compounds and to refute the theory of the presence of the antimicrobial action of arbutin only due to the action of hydroquinone. **Materials and methods.** Molecular docking was performed using AutoDockTools 1.5.6, and antimicrobial activity was assessed using the "well" and "dilution" methods. **Results and discussion.** Theoretical studies have shown that α- and β-arbutin are highly selective inhibitors against gram-negative targets such as deoxyribonucleic acid (DNA) gyrase, dihydrofolate reductase (DHFR), deacetylase, and fungal targets such as 14α-demethylase, beta-1,3-glucanase, thymidylate kinase, whereas hydroquinone had low selectivity against all targets. The "well" assay showed that hydroquinone inhibits gram-positive bacteria more actively than β- and α-arbutin, and in the case of gram-negative bacteria, α-arbutin had a higher inhibitory effect than β-arbutin and hydroquinone, while β-arbutin inhibits fungal growth more actively than α-arbutin and hydroquinone. The minimum inhibitory concentration (MIC) values of hydroquinone for gram-positive, gram-negative and fungal microorganisms were almost 2-3 times higher than MIC of β- and α-arbutin. Meanwhile, MIC of α-arbutin was lower for *E. coli, P. vulgaris* than β-arbutin, and the results were the same in other cases. **Conclusions.** The antimicrobial effect of α- and β-arbutin, hydroquinone against strains of *S. aureus, B. subtilis, E. coli, P. vulgaris, P. aeruginosa* and *C. albicans* was studied *in vitro* and *in silico*. Theoretical results showed that it is impossible to create a "panacea" from one compound that would suppress the growth of both bacteria and fungi. According to theoretical and practical results, the antimicrobial effect of α- and β-arbutin is 2-3 times higher than that of hydroquinone. It has been experimentally confirmed that α-arbutin suppresses the growth of gram-negative strains much more strongly than β-arbutin. The theory that arbutin has an antimicrobial effect only due to the action of hydroquinone has been theoretically and practically refuted.

Keywords: *hydroquinone, hydroquinone glycosides, structure-action relationship, comparative analysis, molecular docking*

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