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BERRY AND GRAPE METABOLITES FOR ANTIMICROBIAL APPLICATIONS AGAINST FOODBORNE BACTERIAL PATHOGENS

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Abstract. The increase in the resistance of microorganisms to chemical substances and conventional drugs presents a serious and obvious problem worldwide, which has determined numerous researches aimed at the identification of new biocides with extended activity. Plants and their derivatives contain a wide variety of secondary metabolites that can inhibit or slow down the growth of bacteria, yeasts and molds. The microbiostatic activity of some berries represents a promising source of alternative solutions for their use in order to reduce the microbial contamination of raw materials and food products. The article elucidates the *in vitro* microbiostatic and microbicidal effects of some berries and grape marc rich in phenolic compounds with microorganisms that cause food spoilage: *Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae*. The composition of the extracts is examined, and possible mechanisms of antimicrobial action are analyzed.

Keywords: *berries, grape marc, phenolic compounds, antimicrobial activity, mechanisms of inhibition of pathogenic bacteria.*

Rezumat. Creșterea rezistenței microorganismelor la substanțele chimice și medicamentele convenționale prezintă o problemă serioasă și evidentă la nivel mondial, ceea ce a determinat numeroase cercetări care vizează identificarea de noi biocide cu activitate extinsă. Plantele și derivații lor conțin o mare varietate de metaboliți secundari care pot inhiba sau încetini creșterea bacteriilor, drojdiilor și mucegaiurilor. Activitatea microbiostatică a unor fructe de pădure reprezintă o sursă promițătoare de soluții alternative pentru utilizarea acestora în vederea reducerii contaminării microbiene a materiilor prime și a produselor alimentare. Articolul elucidează efectele microbiostatice și microbicide *in vitro* ale unor fructe de pădure și tescovină de struguri bogate în compuși fenolici cu microorganisme care provoacă alterarea alimentelor: *Staphylococcus aureus*, *Escherichia coli* și *Klebsiella pneumoniae*. Se examinează compoziția extractelor și se analizează posibilele mecanisme de acțiune antimicrobiană.

Cuvinte cheie: *fructe de pădure, tescovină de struguri, compuși fenolici, activitate antimicrobiană, mecanisme de inhibare a bacteriilor patogene.*

1. Introduction

Antimicrobial-resistant microorganisms present in humans, animals, in food and in the environment is a complex epidemiological problem [1,2]. The epidemiological surveillance system of antimicrobial resistance in the Republic of Moldova is based on the surveillance of the traffic of microbial agents identified from patients and provides only partial and unsubstantial data.

National results on antibiotic susceptibility of pathogenic microorganisms isolated from people show a concerning resistance to compounds included in national protocols for first-line therapy [3]. Primary and secondary multidrug-resistant tuberculosis have high rates of 26% and 64%, respectively, compared to an average of 12% and 50% in the World Health Organization European region. Approximately 60% of strains of microorganisms isolated from patients with surgical wound infections are resistant to antimicrobials. The medication for common diseases such as pharyngitis, bronchitis or food poisoning caused by bacteria fails due to the irrational and excessive use of antimicrobials. The information on the antimicrobial sensitivity of microbial agents shows their significant resistance: every third strain of *Staphylococcus aureus* is resistant to tetracycline (30.4%), clindamycin (35.2%) and erythromycin (38.4%), and approximately two out of three strains of *Streptococcus pneumoniae* are resistant to co-trimoxazole (59.6%), cefaclor (61.3%), oxacillin (64.9%) [4].

Antimicrobial resistance is not exclusively a public health problem, but also an animal health problem with direct economic consequences. The phenomenon of antimicrobial resistance causes a decrease in the effectiveness of antimicrobial treatment in animals, as well as the transmission of resistant bacteria through the food chain and from animals to humans. The concept of antimicrobial resistance also addresses food safety, as antimicrobial-resistant microorganisms and genes spread from animals to humans through the food chain. The emergence of resistant strains of *Salmonella* and *Campylobacter* is caused by the use of antimicrobials in animal husbandry, resulting in cases of human diseases following the consumption of unsafe food, therefore a unified approach to antimicrobial resistance in the world, as well as in the Republic of Moldova, is absolutely necessary [5].

The emergence of multidrug-resistant bacterial strains and the emergence of strains with low sensitivity to antibiotics have led to resurgence of research interests in the discovery of new antimicrobial agents from natural sources that can be used for therapeutic and prophylactic purposes against microbial diseases, such as food preservatives and additives for animal feed. Plants contain a wide range of phytochemicals, which have been traditionally used for centuries traditional medication or ethnomedicines [6].

Thus, the increasing resistance of microorganisms to chemical substances and conventional medicines is a serious and evident problem worldwide, that drives research aimed at identifying new biocides with extended activity. Plants and their derivatives contain a wide variety of secondary metabolites that can inhibit or slow the growth of bacteria, yeasts and molds [7]. The microbiostatic activity of some vegetables represents a promising source of alternative solutions for their use in order to reduce the microbial contamination of raw materials and food products. Berries and plants are an important source of phenolic compounds in the daily diet, and the market for berries has grown over the years due to their contribution to public health. Natural products from berries are being studied as a new arsenal of antimicrobials and prebiotics, due to their ability to selectively inhibit food pathogens, stimulating beneficial microorganisms [8,9]. Berries are traditionally

an important part of the diet. About 50 different berries are grown in the northern regions, and about half of them are edible.

The purpose of this research is to evaluate the microbiostatic effect of the extracts of forest fruits rich in bioactive compounds on microorganisms that cause food spoilage (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae)* in order to explain their microbiostatic action mechanisms.

2. Materials and Methods

2.1. Materials used in research

The plant extracts were obtained from the plant matter of the following fruits native to the Republic of Moldova (RM): white sea buckthorn (*Hippophae rhamnoides* L.), rosehip (*Roza canina* L.), mountain ash (*Sorbus aucuparia* L.), hawthorn (*Crataegus monogyna*), aronia (*Aronia melanocarpa*) and grape marc (*Vitis vinifera* L.) from red varieties.

2.2. Reference strains

Staphylococcus aureus ATCC 25923; *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 obtained from American Type Culture Collection (ATCC), National Agency for Public Health. The reference strains used are cataloged, characterized bacteria with stable, defined antibiotic susceptibility phenotypes. They are used for internal quality control.

2.3. Reagents

Chromatographic purity reagents were used to carry out the research (Sigma-Aldrich (Merck KGaA, Darmstadt, Germany); ethyl alcohol - LC-MS grade Carl Roth (Karlsruhe, Germany).

2.4. Preparation of extracts

To obtain the extracts, the berries were dried at room temperature (20. ± 1.0°C) to a final moisture content of 8.0 ± 1.0%. For extraction, the dry substance was ground and sieved into powder. The extraction process was carried out by two methods: shaking and ultrasound, observing two temperature regimes: 20.0 ± 1.0°C and 45.0 ± 1.0°C and 3 time periods: 0.5h, 1 ,0h and 1.5h [10]. The plant matter to solvent ratio was determined experimentally and depends on the type of plant matter: rosehip (1:15), sea buckthorn and mountain ash (1:12), hawthorn (1:20) and aronia (1:18). To decant the extracts, the obtained samples were centrifuged at 7000 min^{-1} for 10 minutes. The obtained extracts are stored in dark glass bottles at 4°C.

2.5 Analysis of individual bioactive compounds from berries and grape pomace extracts

The tests were performed through the chromatographic method (HPLC, Agilent 1100 Series) [11]. The mobile phase included eluent of 1% CH_3OH (solvent A) and 50% CH_3OH (solvent B), acidified to pH 2.15 with trifluoroacetic acid (TFA). The column system included Security Guard ULTRA HPLC C18 precolumns and C18 100 Å 250×4.6 m columns manufactured by Phenomenex. The injection volume was 20 µL and the detection time was 90 min. Detection was performed at 256, 280, 324 and 365 nm.

The elution gradient was 100% (A) for 10 minutes; 82%(A):18%(B) for the next 10 minutes; 70%(A):30%(B) for 10 minutes; 65%(A):35%(B) for 6 minutes; 40%(A):60%(B) for 15 minutes; 20%(A):80%(B) for 5 minutes; 100%(B) for 15 min and 100%(A) for 10 min.

2.6. Determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of natural compounds

MIC and MBC were determined through the dilution method, which allows to estimate the concentration of the tested antimicrobial agent in the broth (macrodilution or microdilution) [12]. The recorded MIC value is defined as the lowest concentration of the tested antimicrobial agent that inhibits the visible growth of the tested microorganism and is usually expressed in mg/mL. In the study, the MIC and MBC values of the natural antimicrobial agents tested on the selected strains were determined.

2.7. Statistical analysis

All experiments were carried out in three repetitions, and the obtained experimental results were subjected to the usual statistical analysis with the application of descriptive statistics tools (calculation of arithmetic means, standard deviation, coefficient of variation and correlation coefficient). As a test of significance - Student's test, being accepted as statistically true differences [13]. The statistical processing of the results was carried out with the MS Excel program. Statistical significance threshold chosen: $p<0.05$.

3. Results and Discussion

There are various *in vitro* techniques to test minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) in order to determine antimicrobial susceptibility or resistance of microorganisms [14]. The aim of the study was to determine whether the etiological agent is resistant or sensitive to the tested natural antimicrobial agents. The MIC (the lowest concentration that inhibits visible growth of the organism), was determined based on the 90% inhibition level. The antimicrobial agent must inhibit 90% of visible microbial growth. Thus, the tested organism was called "susceptible". Following the tests carried out, it was found that the powders from sea buckthorn and sea buckthorn groats achieve a more pronounced antimicrobial activity against all the pathogenic microorganisms investigated, Table 1.

Table 1

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of plant powders on pathogenic microorganisms

Plants powders	***Staphylococcus aureus* ATCC 25923**		***Escherichia coli* ATCC 25922**		***Klebsiella pneumoniae* ATCC 13883**	
	MIC mg/mL	MBC mg/mL	MIC mg/mL	MBC mg/mL	MIC mg/mL	MBC mg/mL
Sea buckthorn	1.95± 0.12	3.90± 0.23	7.81± 0.37	15.6± 0.7	15.6± 0.5	31.25± 1.25
Sea buckthorn (groats)	15.63± 0.33	31.25± 1.03	62.50± 2.37	125± 5.0	62.5± 2.1	125± 5.0
Aronia	15.63± 0.37	31.25± 0.62	-	-	-	-
Grape pomace	7.81± 0.19	15.62± 0.41	62.50± 1.57	125± 5.0	-	-

Continuation Table 1

Rosehip	3.91± 0.15	7.81± 0.21	31.25± 0.98	62.5± 1.8	62.5± 2.1	125± 5.0
Hawthorn	41.67± 0.56	83.33± 1.23	62.50± 1.87	125± 5.0	-	-
Rosehip (groats)	3.91± 0.23	7.81± 0.29	31.25± 0.71	62.5± 2.5	62.5± 2.1	125± 5.0

Note: Test values performed in triplicate, mean ± standard error, statistical analysis – ANOVA, $\alpha \leqslant 0.05$.

It has been shown that the plant powders with the lowest inhibitory and bactericidal concentration on *Staphylococcus aureus* ATCC 25923 are sea buckthorn, with a minimum inhibitory concentration of 1.95±0.12 mg/mL, followed by rosehip powder and groats (3.91±0.15 mg/mL) and grape pomace (7.81±0.19 mg/mL). In the case of *Escherichia coli* and *Klebsiella pneumoniae* only the white buckthorn powder manifests minimum inhibitory and bactericidal concentrations. Aronia does not show any activity on the Gram negative bacteria studied. Rosehip and hawthorn compounds show weak activity against *Escherichia coli* ATCC 25922, but *Klebsiella pneumoniae* ATCC 13883 is resistant to hawthorn.

The obtained results attest to the fact that, although all the examined powders are extremely rich in biologically active compounds, their direct effects on pathogenic microorganisms depend on several factors. First of all, the bacterial adhesion capacity of biologically active compounds is extremely important, and it depends on their hydrophilic character.

For the microorganisms examined, which are capable of rapidly colonizing meat products and evidently have an increased degree of hydrophobicity, the maximum inhibitory and bactericidal effect was attested in the sea buckthorn and rosehip powder, which have a considerably higher content of biologically active lipophilic compounds (lycopene, β-carotene, zeaxanthin, chlorophylls) than aronia and grape pomace powders, in which flavonoids predominate [15]. At the same time, in hawthorn, the content of biologically active lipophilic compounds is important, but the inhibitory and microbicidal effect was lower than in sea buckthorn and rosehip powder. These results prove that the presence of organic acids and active acidity have an extremely important role since they directly influence bacterial adhesion and the process of inhibiting the proliferation of pathogenic microorganisms.

Sea buckthorn presented antimicrobial effects against gram-positive bacteria such as *Bacillus cereus* and *Staphylococcus aureus*, where *Staphylococcus aureus* showed complete inhibition of bacterial growth at 250 μg/mL, *Bacillus cereus* at 125 μg/mL, *Escherichia coli* at 4 μg/mL and *Pseudomonas aeruginosa* at 300 μg/mL [16]. *Escherichia coli* was found to be more sensitive to *Hippophae rhamnoides* L. berry powder which inhibits it at very low concentration. In another study, researchers analyzed rosehip extract and determined the highest inhibitory activity against 5 strains of Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228) and 5 strains of Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC13883, *Proteus mirabilis* ATCC 35659, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076) [17].

Polyphenols and carotenoids serve as preservatives in food processing. Several studies have shown that antioxidants can act in various ways, including as free radical

scavengers or chelators, preventing lipid oxidation and thereby preventing nutrient loss and inhibiting the potential formation of toxic compounds [18,19]. Food spoilage and food poisoning caused by the growth of pathogenic bacteria are major problems in the food industry. There is a growing interest in the use of active natural preservatives. Thus, research into the mechanisms of antimicrobial and antioxidant action of bioactive plant compounds is of particular interest.

The effects of natural antioxidants depend on the absorption of phenolic hydrogen in radical reactions, the stability of the natural antioxidant radical formed during radical reactions, and the substituents present in the structure. An antioxidant is a molecule that reduces or prevents the oxidation of other chemicals [20]. Oxidation is part of a redox reaction and consists in the transfer of electrons from a substance to an oxidizing agent. This reaction can produce free radicals, which cause destructive chain reactions. Antioxidants are able to stop these chain reactions by oxidizing free radicals and thus blocking their action. These properties are particular to several families of chemical compounds: thiols, phenols, carotenoids, etc.

Table 2 shows the composition of individual polyphenols, identified in hydroethanolic berries and grape pomace extracts (HPLC method) [21, 22].

Sea buckthorn hydroethanolic extracts contain significant amounts of salicylic acid (24.48 mg/100 mL), hyperoside (38.53 mg/100 mL), ferulic acid methyl ester (25.43 mg/100 mL), polydatin (5.4 mg/100mL), ferulic acid (2.19 mg/100 mL), chlorogenic acid (1.43 mg/100mL), cis-resveratrol (4.17 mg/100mL) and trans-resveratrol (1.2 mg/100 mL). Rosehip extracts have important amounts of substances, such as derivatives of hydroxybenzoic acid (salicylic, gallic, protocatechuic), hydroxycinnamic acid (ferulic), flavones (catechin, epicatechin), flavonoids (procyanidin B2 and procyanidin B1), and the methyl ester of ferulic acid. The main phenolic compounds detected in aronia extract were catechin (15.41 mg/100 mL), epicatechin (4.7 mg/100 mL), ferulic acids (5.51 mg/100 mL), salicylic (2.65 mg/ 100 mL), protocatechuic acid (1.88 mg/100 mL), polydatin (1.27 mg/100 mL), ferulic acid methyl ester (1.48 mg/100 mL), but also gallic, *para*- and *meta*-benzoic acids, procyanidin B1 and B2. Grape pomace extracts contain significant amounts of procyanidin B2, gallic acid, catechin, procyanidin B1, ferulic acid and its methyl ester.

Table 2

Individual polyphenols identified in hydroethanolic berries and grape pomace extracts

Polyphenols	Sea buckthorn, mg/100mL	Rosehip, mg/100mL	Aronia, mg/100mL	Grape pomace, mg/100mL
Gallic acid	0.16±0.01	0.85±0.01	0.39±0.01	1.95±0.01
m-hydroxybenzoic acid	0.020±0.002	0.020±0.001	0.13±0.01	0.010±0.002
Protocatechuic acid	0.98±0.01	0.43±0.01	1.88±0.01	0.32±0.01
p-hydroxybenzoic acid	0.21±0.01	0.19±0.01	0.21±0.01	0.34±0.01
Gentisic acid	0.15±0.01	0.27±0.01	-	-
Vanillic acid	0.17±0.01	0.13±0.01	0.09±0.01	-

Continuation Table 2

Salicylic acid	24.48±0.05	1.07±0.01	2.65±0.02	-
Syringic acid	-	-	0.05±0.01	0.19±0.01
p-coumaric acid	0.010±0.002	0.010±0.001	0.06±0.01	-
Ferulic acid	2.19±0.01	0.32±0.01	5.51±0.03	0.82±0.01
Caffeic acid	0.006±0.001	-	0.09±0.01	-
Sinapic acid	0.13±0.01	-	0.08±0.01	0.008±0.001
Catechin	-	2.05±0.01	15.41±0.15	1.34±0.01
Epicatechin	0.37±0.01	0.49±0.01	4.7±0.02	-
Quercetin	0.030±0.005	0.020±0.001	-	0.19±0.01
Hyperoside	38.53±0.02	0.41±0.01	0.97±0.01	0.37±0.01
Procyanidin B1	0.19±0.01	0.70±0.01	0.27±0.01	1.33±0.01
Procyanidin B2	0.10±0.01	1.75±0.01	0.12±0.01	15.34±0.15
Chlorogenic acid	1.43±0.02	-	-	-
Polydatine	5.40±0.01	0.06±0.01	1.27±0.01	-
Trans-resveratrol	1.20±0.01	-	0.005±0.001	-
Cis-resveratrol	4.17±0.01	0.010±0.001	0.011±0.001	-
Methyl ester of ferulic acid	25.43±0.02	1.44±0.01	1.48±0.02	0.74±0.01

Note: results are presented as mean ± standard deviation.

Thus, it was found that the extracts used are sources rich in bioactive substances. Numerous bibliographic studies demonstrate their antimicrobial effect [23-26]. A special role in combating antibiotic resistance is attributed to natural bioactive compounds [27].

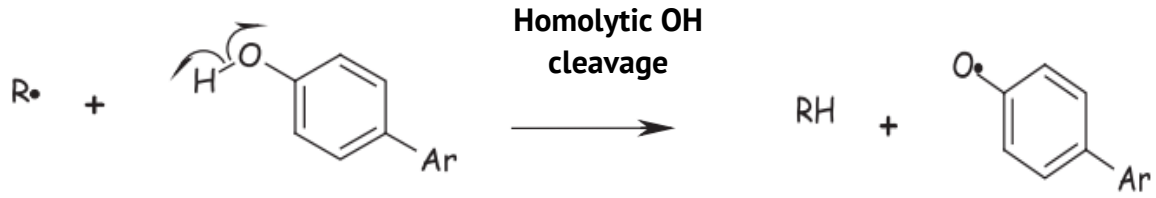
The molecular effects responsible for the antioxidant properties of polyphenols are recognized through three main mechanisms, arising from direct reaction with free radicals and from free metal chelation, the latter involved in reactions ultimately generating free radicals, Figure 1 [28].

$$ArOH + R\bullet \rightarrow ArO\bullet + RH \quad (1)$$

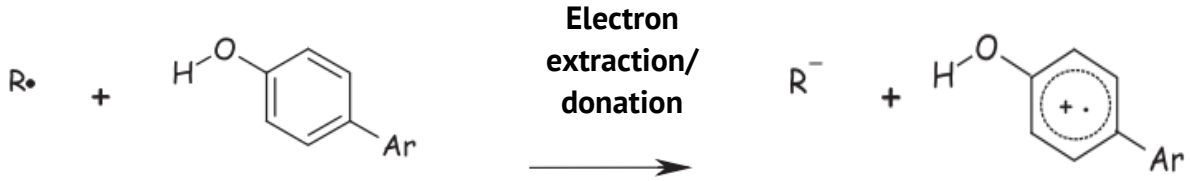
As primary antioxidants, polyphenols inactivate free radicals according to hydrogen atom transfer (HAT) (1) and electron transfer (SET) mechanisms (2) [29]. In mechanism 1, the antioxidant, ArOH, reacts with the free radical R by transferring a hydrogen atom to it by homolytic cleavage of the O–H bond.

The products of the reaction are the harmless species RH and the oxidized ArO• radical, Figure 1. Even if the reaction leads to the formation of another radical, it is less reactive than R, being stabilized by several factors.

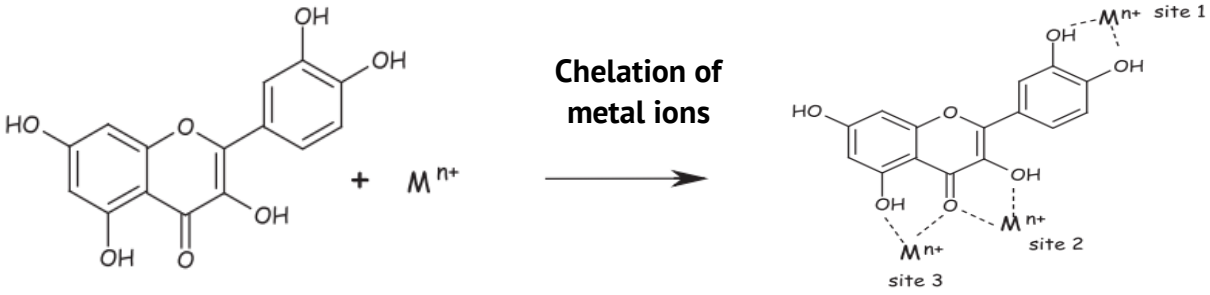
The bond dissociation enthalpy (EDL) of the phenolic O–H bond is an important parameter in the evaluation of antioxidant action.



(A) Hydrogen atom transfer (HAT) (1)



(B) Electron transfer (SET) (2)



(C) Chelation of transition metals

Figure 1. Mechanisms responsible for the antioxidant properties of polyphenols [28].

The lower the EDL value, the easier the dissociation of the phenolic O–H bond and the reaction with the free radical.

The SET mechanism (2) provides for the donation of an electron to the R• radical:

$$ArOH + R\bullet \rightarrow ArOH\bullet^{+} + R^{-} \quad (2)$$

The anion R^{-} is an energetically stable species with an even number of electrons, while the cationic radical $ArOH\bullet^{+}$ presents less reactive radical species, especially since polyphenols are aromatic structures in which the odd electron, originating from reactions with free radicals, has the possibility of being distributed throughout the molecule, resulting in radical stabilization (Figure 3.15 B) [30]. In the SET mechanism, the ionization potential is the most significant parameter for evaluating the trapping activity. The lower

the ionization potential, the easier it is to withdraw/donate electrons and react with free radicals.

Another antioxidant mechanism consists in the chelation of transition metals, capable of generating free radicals. Transition metal ions can be chelated by polyphenols, leading to stable complex compounds, Eq. (3) [31]. Some metals in their reduced oxidation state (mainly the Fe^{2+} ion) can be involved in Fenton reactions, resulting in highly dangerous reactive oxygen species (ROS) [32]:

$$H_2O_2 + M^{n+} \rightarrow HO^- + HO\bullet + M^{(n+1)+} \quad (3)$$

HO• is generally accepted as one of the most reactive radicals. It has a very short half-life (about 10^9 s) and very high reactivity. Hydroperoxides are metabolized by superoxide dismutase, but hydroxyl radicals cannot be eliminated by enzymatic reactions.

Transition metals such as copper, manganese, cobalt are able to catalyze this reaction, under certain conditions when the ions of these metals are not bound to proteins or chelators. Similar reactions can cause a specific accumulation of free radicals, which initiate the processes of damage to biomolecules. Metal chelating agents lower their redox potentials rendering them inactive. Furthermore, natural metal chelators such as flavonoids show beneficial effects on the body, while synthetic chelators may present some toxicity issues.

Within the electron transfer mechanism, the most efficient are the compounds that show planar conformation and extensive electronic delocalization, so that the values of the ionization potentials are lower than those of the reference phenol, as occurs in tocopherol, reseveratrol, quercetin. Most polyphenols appear to eliminate free radicals through the hydrogen atom transfer mechanism, since the single electron transfer process involves high energy levels. Flavonols show a stronger antiradical effect than the corresponding flavones due to the presence of the 3-hydroxyl group. The most acidic polyphenolic compounds are those characterized by a high degree of p-electron delocalization, for which deprotonation gives way to anionic species stabilized by resonance phenomena, their stability being increased by the presence of a hydrogen bond formation pattern.

Polyphenols are able to chelate transition metals via multiple OH groups and the carbonyl fragment. The flavonol quercetin, for example, can form stable complexes with Fe^{2+} and Cu^{2+} cations in both neutral and ionized form. Among the possible chelating sites, the 3-OH/4-keto and 5-OH/4-keto positions show the highest complexation capacity, while catechol appears to be a weak chelating agent.

The mechanisms of antibacterial activity of polyphenolic antioxidants are still not fully explored and several possible pathways of molecular mechanisms are proposed [33,34]. The chemical diversity of antioxidants, as well as studies conducted not on single, extracted substances, but on extracts containing several classes of compounds, make it difficult to clearly identify the molecular mechanisms responsible for inhibiting the growth of microorganisms and their death. Three main mechanisms of action of polyphenols are proposed: (a) inhibition of cytoplasmic membrane function; (b) inhibition of nucleic acid synthesis; (c) inhibition of energy metabolism [35]. Polyphenols change the morphology of bacterial cells, damage the cell wall, trigger the leakage of intracellular material. The relationship between chemical structure and antibacterial activity of antioxidants is related to the number and position of hydroxyl and methoxyl groups in the antioxidant structure. A structure-activity relationship study showed that the antimicrobial activity of polyphenols is

associated with the hydrophobic and amphiphilic nature of the molecule and the OH group in position 3 of the C ring [36]. It is considered that Gram-negative bacteria are more resistant to the effects of antioxidants, due to the presence of the lipophilic outer membrane formed by phospholipids, which makes the cell wall of these bacteria impermeable. Moreover, it is believed that Gram-negative bacteria can chemically degrade polyphenols by their enzymes [37].

One of the mechanisms of antimicrobial action of antioxidants consists in their interaction with cell wall proteins, which has the effect of damaging bacterial membranes and their permeabilization, loss of chemiosmotic control, leakage of intracellular components and eventual cell death [37,38]. Other recent studies emphasize the inhibitory properties of polyphenols in relation to biofilm production by bacteria [39]. Polyphenolic compounds appear to induce endogenous oxidative stress in bacterial cells and lead to the formation of ROS. It has been shown that ROS generated in bacterial cells are responsible for the oxidative damage of fatty acids in bacterial membranes and ultimately lead to the death of pathogens [40].

The microbiological activity of polyphenols also results from the ability of these compounds to influence the biosynthesis of proteins that are necessary for the proper functioning of bacteria. Antioxidant-dependent changes in bacterial proteins involved in, for example, energy metabolism and the tricarboxylic acid cycle, DNA metabolism and fatty acid biosynthesis lead to irreversible changes in bacterial metabolism and eventual death [40]. An important property in the context of the antibacterial activity of polyphenolic compounds is their ability to inhibit the enzyme DNA gyrase, which, in turn, leads to the inhibition of bacterial DNA synthesis [42,43]. Another enzyme essential for the energy metabolism of bacteria is ATP synthase, which undergoes a polyphenol-dependent inhibition, leading to the death of microorganisms [44,45].

5. Conclusions

The results of microbiological tests showed that different bacterial species have different antimicrobial sensitivities to the tested natural powders. In general, it has been observed that Gram positive bacteria (*Staphylococcus aureus*) to be more sensitive than Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*). As a result of the tests carried out, it was found that the sea buckthorn and the sea buckthorn groats powder achieve a pronounced MIC against all the pathogenic microorganisms investigated, predominantly on the Gram-positive microorganisms. Rosehip powder possesses very high antimicrobial activity against *Staphylococcus aureus* ATCC 25923. Gram-negative bacterial strains are less sensitive to the effect of berry powders. The antimicrobial activity of hawthorn is very low and *Klebsiella pneumoniae* is resistant.

The analyzed extracts contain significant amounts of bioactive compounds, belonging to different classes of polyphenols, especially derivatives of hydroxybenzoic acid (salicylic, gallic, protocatechuic), hydroxycinnamic acid (ferulic), flavones (catechin, epicatechin), flavonoids (procyanidin B2 and procyanidin B1) and esters. The molecular effects responsible for the antioxidant and antimicrobial properties of polyphenols were examined. Most polyphenols seem to eliminate free radicals through the hydrogen atom transfer mechanism, deprotonation gives way to anionic species stabilized by resonance phenomena, their stability being increased by the presence of a hydrogen bond formation pattern. Polyphenolic compounds induce endogenous oxidative stress in bacterial cells and lead to the formation of ROS. This leads to damage to bacterial membranes and their

permeabilization, loss of chemiosmotic control, leakage of cytoplasmic components, which produces cell death.

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