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ANTIMICROBIAL ACTIVITY OF ROSE HIP AND HAWTHORN POWDERS ON PATHOGENIC BACTERIA

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Abstract. In this present study, rose hip and hawthorn powders were investigated against three pathogenic bacteria strains: *Staphylococcus aureus*, *Escherichia coli* și *Klebsiella pneumoniae*. The chemical composition and antiradical activity of the plant extracts were investigated. The antiradical activity of the hydroalcoholic extracts of rose hip is 85.11 ± 0.02 and hawthorn - 80.27 ± 0.23% DPPH inhibited, which correlates with the high content of polyphenolic and flavonoid compounds. The content of β-carotene and lycopene in the liposoluble extracts of rose hip is 14.85 times and 28.83 times higher than in hawthorn extracts. The rose hip powders show an antimicrobial activity pronounced to *Staphylococcus aureus*. In the case of *Escherichia coli* and *Klebsiella pneumoniae* antimicrobial activity of hips is on average 1.3 times higher than that hawthorn powders. The rose hip powders showed the best inhibitory activity against *Staphylococcus aureus*, Gram-positive bacteria, followed by *Escherichia coli* and *Klebsiella pneumoniae* - Gram-negative bacteria. The investigated plant powders have shown promising antimicrobial potential against pathogenic microorganisms and can be used in the food industry to reduce the microbial contamination of raw materials and food.

Keywords: *rosehip, hawthorn, pathogenic bacteria, chemical composition, antimicrobial activity, food safety.*

Introduction

At present, an important concern for public health is food poisoning, which results from the consumption of food contaminated with pathogenic bacteria. The Food and Agriculture Organization of the United Nations (FAO) has reported that diseases due to contaminated food are the most widespread health problem in the world and an important cause of economic productivity decline [1]. *Staphylococcus aureus* and *Escherichia coli* present the most frequent causes of the mass diseases of the population caused by contaminated food [2]. *Klebsiella pneumoniae* belongs to the *Enterobacteriaceae* family is a pathogen, which is responsible for nosocomial, urinary, respiratory tract and blood infections. Following manipulations, raw material and food can be contaminated with this type of pathogenic bacteria [3]. In this context, worldwide researchers are concerned with identifying and evaluating antimicrobial agents to inhibit the development of these pathogenic bacteria in food, in order to ensure consumers with safe and healthy food.

With the increase in bacterial resistance to antibiotics, there is a particular interest in investigating the antimicrobial effects of natural bioactive compounds from plant, such as essential oils and extracts, against pathogenic bacteria, for the preservation of food by harmless methods to the health of the consumer [4]. The essential oils and plant extracts have an antimicrobial effect with low toxicity and can be recommended as potential natural preservatives. The possibilities of using plant extracts as natural antibiotics in food have been studied to extend their shelf life [5].

The analysis of bibliographic sources has shown that the antimicrobial effect of plants is due to their chemical composition, namely the presence of polyphenolic compounds, carotenoids, flavonoids, vitamins, etc. [6]. It is known that polyphenolic compounds, being in optimal combination, have a stronger impact on pathogenic microorganisms than on their own [4].

The aim of this study was to investigate antimicrobial activity, minimal inhibitory and bactericidal concentrations of rose hip and hawthorn powders on pathogenic microorganisms.

Materials and methods

The autochthonous fruits of rose hip (*Rosa Canina*) and hawthorn (*Crataegus monogyna*) were used for research. The hips and hawthorn were washed and dried at 65±1°C to a humidity of 8.0±0.25%, were ground to the powder and sieved. The granulation of the powder after sieving was 140±10μm.

The obtained powders were subjected to hydroalcoholic extraction using 50% vol. ethanol. The extraction was carried out in a solid-liquid ratio of 1:15 in the case of the fruits of the rose hip and 1:20 in the case of hawthorn fruits in the water bath at 45±1°C for 1 hour under stirring of 60 min^{-1}. The obtained extract was filtered and stored in dark-coloured containers at 5.0±1.0°C [7]. In the hydroalcoholic extracts of rose hip and hawthorn powders were determined the content of tannins [8], the content of polyphenolic compounds [8] and antiradical activity [9].

The extraction of the liposoluble fraction was carried out in a solid-liquid ratio of 1:15 in the case of fruit and in the ratio of 1:20 in the case of hawthorn fruits in a water bath at 45.0 ± 0.1°C for 1.5 h under stirring of 60 min^{-1}. As an organic solvent was used refined and deodorized sunflower oil „Floris”. The obtained extract was filtered and stored in dark-coloured containers at 5.0±1.0°C [10]. The content of β-carotene and lycopene [11], antiradical activity [9] were determined in liposoluble extracts.

The antimicrobial activity of the plant powders was determined by the agar diffusion test [12].

Antibacterial action was determined by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) [13]. By the ratio MBC/MIC, antibacterial activity was assessed. If the ratio MBC/MIC=1 or 2, the effect is bactericidal. If the ratio MBC/MIC=4 or 16, the effect is bacteriostatic [14].

The variance analysis of the results was carried out by least square method with application of Student test and Microsoft Office Excel program version 2010. The differences were considered statistically significant if probability was greater than 95% (p-value <0.05).

All assays were performed in triplicate at room temperature 20±1°C. The experimental results are expressed as average ± SD (standard deviation).

Results and discussions

It is known that there is a correlation between the chemical composition of the bioactive compounds and the antimicrobial activity of the plant matter [15]. Table 1 shows the content of biologically active compounds and antiradical activity in hydroalcoholic extracts from rose hip and hawthorn fruits.

The antiradical activity of the hydroalcoholic extracts of hips constitutes 85.11 ± 0.02% DPPH inhibited and hawthorn extracts - 80.27 ± 0.23% DPPH inhibited. In the case of hip extracts, the antiradical action correlates with the high content of polyphenolic and flavonoid compounds [16], since vitamin C-free extracts can still show considerable antioxidant activity [17].

This action has a protective effect against oxidative stress, increasing the activity of antioxidant enzymes such as superoxide-dismutase and catalase [18]. In the case of hawthorn extracts, the antioxidant activity is due to the presence of several groups of phenolic compounds such as epicatechin, aglycons, glycosides such as B-type oligomeric procyanidins, flavonols, phenolic acids, C-glycosyl flavones [19].

Table 1

The content of biologically active compounds and antiradical activity in hydroalcoholic extracts from rose hip and hawthorn fruits [7, 20].

Plant powders	Tannins (mg TAE·g^{-1} DW)	Total phenols (mg GAE·g^{-1} DW)	Antiradical activity (% of inhibition of DPPH)
Rose hip	106.41±1.34	26.98±0.36	85.11±0.02
Hawthorn	13.67±0.02	3.83±0.01	80.27±0.23

Note. The results are presented as means ± standard deviations of three experiments.

The results in Table 1 show that the tannin content and polyphenol content in the hip extracts are 7.78 times and 7.04 times higher than in hawthorn extracts. These can be explained by the fact that plant's genotype and development stages have a strong impact on the chemical composition of the studied fruits [21].

The content of liposoluble bioactive compounds and antiradical activity in extracts of rose hip and hawthorn fruits is shown in Table 2.

Table 2

The content of ß-carotene, lycopene, zeaxanthin and antiradical activity in liposoluble extracts of rose hip and hawthorn fruits [20]

Plant powders	Content of ß-carotene (mg·L^{-1} extract)	Content of lycopene (mg·L^{-1} extract)	Content of zeaxanthin (mg·L^{-1} extract)	Antiradical activity (% of inhibition of DPPH)
Rose hip	17.08±0.05	18.45±0.15	19.12±0.07	62.98±2.43
Hawthorn	1.15±0.02	0.64±0.01	nd	51.98±3.39

Note. The results are presented as means ± standard deviations of three experiments; nd - no detected

The results in Table 2 indicate that the rose is rich in carotenoids relative to the hawthorn, which has a low content. Thus, the content of β-carotene and lycopene in the rose hip extracts is 14.85 times and 28.83 times higher than in hawthorn extracts. Zeaxanthin in hawthorn extracts was not detected. Carotenoids have a property to protect the human body against free radicals and reduce the risk of cancer and cardiovascular disease [22]. The antiradical activity of the examined liposoluble extracts is on average 1.44 times lower than that of hydroalcoholic extracts. This phenomenon can be explained by the diversification of water-soluble bioactive compounds, which have antioxidant properties.

In this present study, three bacteria strains (Gram-negative and Gram-positive bacteria) were used. Table 3 shows the antimicrobial activity of rose hip and hawthorn powders on pathogenic microorganisms: *Staphylococcus aureus, Escherichia coli* și *Klebsiella pneumoniae*.

The results in Table 3 show that the hip powders have an antimicrobial activity more pronounced on *Staphylococcus aureus*, the diameter of the inhibition zone being 16 ± 1 mm. In the case of *Escherichia coli* and *Klebsiella pneumoniae* microorganisms, the antimicrobial activity of rose hip powders is on average 1.3 times higher than that of hawthorn powders.

Table 3

Inhibition zone diameters (mm) recorder in agar diffusion test using Rose hip and Hawthorn powders

Plant powders	***Staphylococcus aureus* ATCC *25923***	***Escherichia coli* ATCC *25922***	***Klebsiella pneumoniae* ATCC 13883**
Rose hip	16±1	10±1	9±1
Hawthorn	10±1	8±1	7±1

Note. The results are presented as means ± standard deviations of three experiments.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the plant powders analyzed on pathogenic microorganisms capable of causing food contamination are shown in Table 4.

Table 4

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and bactericidal effect of plant powders

Bacteria	**MIC (mg·mL^{-1})**	**MBC (mg·mL^{-1})**	**MIC / MBC**	**Bactericidal effect**
		Rose hip powder		
Staphylococcus aureus ATCC 25923	3.91±0.15	7.81±0.21	2	+
Escherichia coli ATCC 25922	31.25±0.98	62.5±1.8	2	+
Klebsiella pneumoniae ATCC 13883	62.5+2.1	125±5	2	+

Continuation Table 4

Hawthorn powder				
Staphylococcus aureus ATCC 25923	41.66±1.35	83.33±2.47	2	+
Escherichia coli ATCC 25922	62.5±2.2	125±5	2	+
Klebsiella pneumoniae ATCC 13883	nd	nd	nd	nd

Note. The results are presented as means ± standard deviations of three experiments.
nd: no detected activity; "+": bactericidal effect.

The results in Table 4 demonstrate that the rose hip powders have the smallest inhibitory and bactericidal concentrations on all pathogenic microorganisms investigated. The rose hip and hawthorn powders showed the bactericidal effect on all pathogenic microorganisms investigated except *Klebsiella pneumoniae* to which hawthorn powders not exhibited the bactericidal effect.

Plants are the largest source of natural antimicrobial agents. They produce very bioactive molecules that can interact with other organisms in their environment. Many of these compounds act against pathogenic bacteria [23].

Analysis of bibliographic data indicates that bioactive compounds, such as polyphenols, are responsible for antimicrobial activity, inhibiting several types of microorganisms [6]. It is well known that phenol is a strong chemical antiseptic [24]. In bibliographic sources, the antimicrobial activities of phenolic compounds are well documented [25].

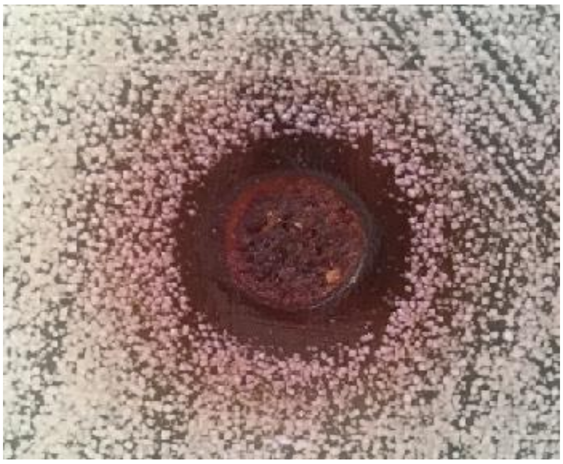
Figures 1 and 2 show the antimicrobial action of rose hip and hawthorn powders on pathogenic bacteria strains.

Polyphenols, such as flavonoids and tannins, show significant antibacterial activity [26]. The antimicrobial activity of flavonoids is due to their ability to form complexes with the extracellular and soluble proteins of the cell walls of the bacteria. In the case of tannins, antimicrobial activity may be related to their ability to inactivate microbial enzymes and proteins inside the cells of microorganisms [27].

It has been observed that rose hip powders have the best inhibitory activity against *Staphylococcus aureus*, being Gram-positive bacteria, followed by *Escherichia coli* and *Klebsiella pneumoniae* - Gram-negative bacteria. The sensitivity of Staphylococcus aureus microorganisms may be due to the cell wall structure and outer membrane [28].

Gram-positive bacteria are more sensitive to the action of plant powders than Gram-negative bacteria [29]. This phenomenon can be explained by important differences in the outer layers of Gram-positive bacteria. The high level of phospholipids reduces the cell wall permeability of Gram-negative microorganisms compared to gram-positive bacteria.

At the same time, Gram-negative bacteria have an outer membrane and a periplasmic space, not found in Gram-positive bacteria [30]. The hydrophilic surface of the outer membrane of the bacteria, which consists of lipopolysaccharide molecules, forms a resistance of Gram-negative bacteria to antibacterial substances.



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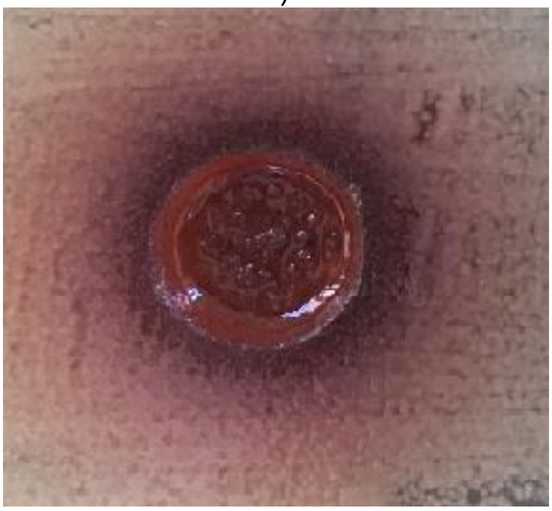

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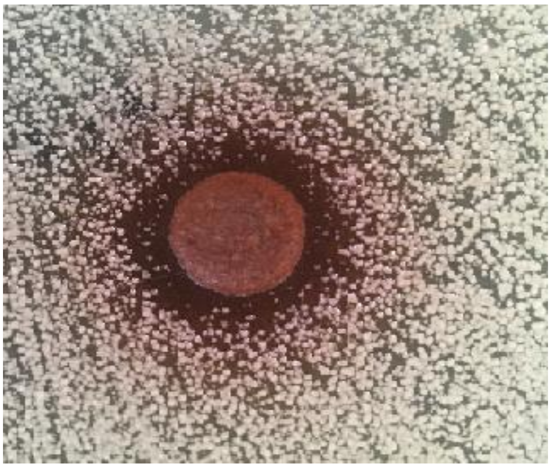
Figure 1. The antimicrobial action of rose hip powders on: a) *Staphylococcus aureus*; b) *Escherichia coli*; c) *Klebsiella pneumonia.*



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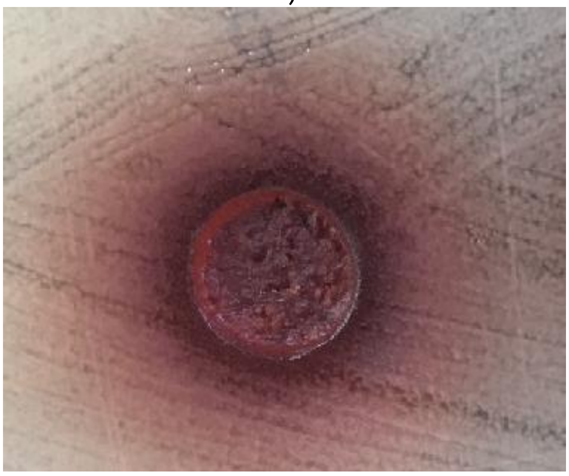

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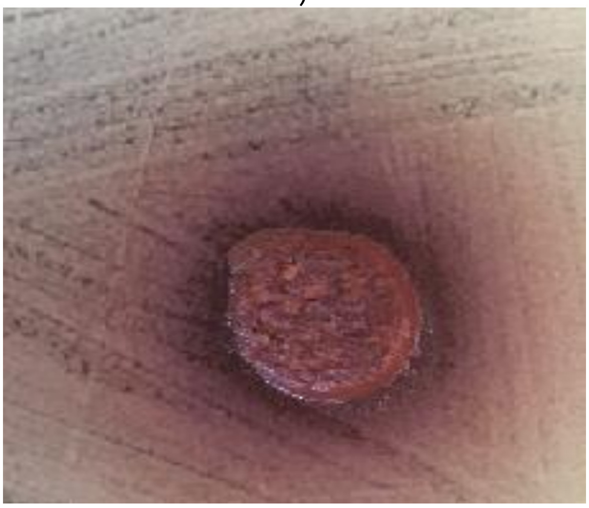

c)

Figure 2. The antimicrobial action of hawthorn powders on: a) *Staphylococcus aureus*; b) *Escherichia coli*; c) *Klebsiella pneumonia.*

Lipopolysaccharide molecules present a barrier to the penetration of numerous antibacterial molecules and are associated with enzymes from periplasmic space that are capable of breaking the molecules introduced from the outside [31]. Thus, rose hip and hawthorn powders, having an antimicrobial potential against pathogenic microorganisms, can reduce the degree of microbial contamination of raw materials and food.

Conclusions

To decreasing the contamination of raw and food products with pathogenic microorganisms that cause food poisoning, the rose hip and hawthorn powders were investigated.

The antiradical activity of the hydroalcoholic extracts of rose hip is 85.11 ± 0.02 and hawthorn - 80.27 ± 0.23% DPPH inhibited, which correlates with the high content of polyphenolic and flavonoid compounds. The content of β-carotene and lycopene in the liposoluble extracts of rose hip is 14.85 times and 28.83 times higher than in hawthorn extracts. The antiradical activity of the liposoluble extracts of the examined fruits is on average 1.44 times lower than the hydroalcoholic extracts.

The rose hip powders show a pronounced antimicrobial activity against *Staphylococcus aureus*. In the case of *Escherichia coli* and *Klebsiella pneumoniae* antimicrobial activity of hips is on average 1.3 times higher than that hawthorn powders. The rose hip powders have the smallest inhibitory and bactericidal concentrations on all pathogenic microorganisms investigated. The plant powders showed the bactericidal effect on all pathogenic microorganisms investigated except *Klebsiella pneumoniae* to which hawthorn powders not exhibited the bactericidal effect. The rose hip powders showed the best inhibitory activity against *Staphylococcus aureus*, Gram-positive bacteria, followed by *Escherichia coli* and *Klebsiella pneumoniae* - Gram-negative bacteria. The sensitivity of Gram-positive microorganisms is due to the structure of the cell wall and the absence of an outer membrane.

The investigated plant powders have shown promising antimicrobial potential against pathogenic microorganisms and can be used in the food industry to reduce the microbial contamination of raw materials and food.

Acknowledgments

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