



Article Bioactive Substances and Microbiological Quality of Milk Thistle Fruits from Organic and Conventional Farming

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Abstract: The agricultural policy of the European Union is currently focused on increasing the area of organic farming. Medicinal plants, including milk thistle (Silybum marianum [L.] Gaertn.), are particularly suitable for this type of cultivation. The aim of this study was to compare milk thistle fruits from organic and conventional farming in terms of the content of silymarin and individual flavonolignans, oil content, microbiological purity, as well as antimicrobial activity of the silymarin extract, mainly in relation to microorganisms responsible for skin infections. The raw material of Silybi mariani fructus obtained from organic farming did not differ in terms of silymarin and oil content compared to the raw material from conventional cultivation. However, it differed in the composition of silymarin and the level of microbiological contamination. Raw material from organic farming was mostly characterized by a higher proportion of the sum of silydianin and silychristin in the silymarin complex than the sum of silvbinin A and silvbinin B. In the samples from conventional cultivation, only genotypes with a predominance of silybinins were present. Although the total number of microorganisms (TAMC) and yeasts and molds (TYMC) on fruit from organic farming were several times higher than on fruit from conventional farming, it was still within the standards set for food products. All raw materials were free of Escherichia coli, Salmonella spp. and Listeria monocytogenes. In addition, it was shown that the silymarin extract from organic farming was generally characterized by greater antimicrobial activity, especially in relation to Staphylococcus aureus, which is resistant and troublesome in skin infections.

Keywords: Candida; milk thistle; MRSA; silybinin; silydianin; Staphylococcus aureus

1. Introduction

Organic farming is the most pro-environmental method of agricultural production. This is a global trend that is a response to the growing awareness of consumers and the changing structure of demand on the market. Currently, the European Commission has set out an ambitious action plan for the further development of organic production by member states towards 25% of organic agricultural area by 2030 [1]. In European organic farming, the largest area of arable land is occupied by cereals [2]. Herbal (medicinal) plants, although they have a small percentage of the sown area, due to their properties and the possibility of cultivation without synthetic fertilizers and pesticides, perfectly fit into the idea of organic farming. Moreover, herb cultivation can significantly increase the economic efficiency of organic farms, especially since herbal companies are systematically expanding their offer with certified organic products.



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Milk thistle (*Silybum marianum* [L.] Gaertn.) of the family Asteraceae belongs to a group of species that occupy one of the leading positions among medicinal plants, especially in Poland, Slovakia, and the Czech Republic. In Poland, the areas of milk thistle cultivation range from 1500 to 2000 ha. The herbal raw material of milk thistle are dry fruits (*Silybi mariani fructus*) which in the fruit cover accumulate unique active substances such as flavonolignans (silybinin A, silybinin B, isosilybin A, isosilybin B, silychristin, silydianin). The complex of these secondary metabolites is known as silymarin and is used in the production of medicines and dietary supplements intended mainly for people with liver ailments and diseases. However, the use of the herbal raw material of milk thistle and the use of whole plants is much wider and includes: culinary applications (cold-pressed oil rich in essential fatty acids (EFA), fruit additions to bread, edible sprouts), self-medication (consumption of defatted or non-defatted ground fruit as a source of silymarin), the use of fruits or whole plants in animal nutrition, production of cosmetics and bioenergy, soil phytoremediation [3].

A wide range of applications makes this plant very attractive for organic farms, as well as herbal companies processing such raw materials. However, the rules of organic farming differ fundamentally from the rules of conventional farming. Obtaining certification is conditioned primarily by the use of certified seeds and the non-use of synthetic mineral fertilizers and synthetic plant protection products [4]. However, the use of organic fertilizers and organic material can increase soil organic carbon and nutrients, and enhance crop yields [5]. These important elements of agricultural technology can also change the quality of herbal raw material, which is extremely important for products with medicinal or food values. The number of scientific studies on milk thistle cultivation and properties is relatively extensive, but the issues related to organic production are only fragmentary. They usually concern only selected elements of agricultural technology related to organic production, e.g., the impact of the use of organic fertilizers and some amendments on the yield and quality of the milk thistle raw material [6,7].

Scientific studies report that milk thistle fruits within the cultivars and individual plants differ in color, 1000 seed weight, germination capacity, and also in the content of fat and silymarin [8–11]. The proportion of individual flavonolignans in silymarin, similarly to the proportion of fatty acids in the oil, is genetically determined [8,11]. However, the content of silymarin, and to a lesser extent also the content of oil in milk thistle fruit is shaped by environmental and cultivation conditions. In a temperate climate, higher temperatures and a long growing season favor the accumulation of silymarin [7,12,13].

Silymarin, besides its well-known hepatoprotective properties, has also been shown to have antioxidant, antifibrotic, anti-inflammatory, choleretic, and immune-stimulating, regenerative, cytoprotective, cardioprotective, neuroprotective, anti-carcinogenic properties [14]. Recently, attention has also been paid to antimicrobial properties, especially in the context of the emergence of many antibiotic-resistant pathogens [15]. The importance of silymarin in the treatment of skin infections is also beginning to be appreciated [16-18]. The course of these infections is often long-lasting, and the use of synthetic drugs, including antibiotics, is not very effective. It appears that biologically active substances obtained from plants have as good or even better effects compared to synthetic drugs, and, in addition they do not cause microbial resistance, which often occurs during antibiotic therapy. Among the bacteria, Staphylococcus aureus is the most common cause of skin infections, including impetigo and cellulitis as well as ulcers and abscesses [19]. Increasingly, however, the cause of these infections is identified as a multidrug-resistant strain of MRSA (methicillin-resistant Staphy*lococcus aureus*), insensitive to commonly used antibiotics [20,21]. In addition, emerging Gram-negative pathogens can cause fatal skin and soft tissue infections which is also a major threat [22]. Among the pathogenic yeasts, *Candida albicans* is most often isolated from skin lesions; however, new species are emerging among *Candida* genus that show less sensitivity and more resistance to antifungal drugs (e.g., C. auris, C. parapsilosis, C. krusei, C. glabrata, and C. tropicalis) [23,24]. In light of the above data, it seems reasonable to use natural antimicrobial substances, e.g., silymarin, for the production of ointments and/or creams.

The aim of this study is to compare milk thistle fruits from organic and conventional crops in terms of the content of silymarin and individual flavonolignans, oil content, microbiological purity, as well as antimicrobial activity of the silymarin extract, mainly in relation to microorganisms responsible for skin infections.

2. Materials and Method

Milk thistle fruits came from five organic and five conventional crops grown in temperate climate conditions. Three organic crops of unknown genotypes were located in Poland, the Mirel cultivar was grown in the Czech Republic, and the Silyb cultivar in Slovakia. The Silma cultivar and an unknown genotype were grown in two conventional crops in Poland, the Silma and Silyb cultivars were cultivated in Slovakia, and the Mirel cultivar in the Czech Republic (Table 1). Milk thistle was cultivated on medium-compact soils with a pH of 6.0–6.2 The average annual temperature in Poland is 9.5 °C, in Slovakia 14 °C, in the Czech Republic 13 °C, and the sum of precipitation in lowland areas in Poland is 500–600 mm, and in Slovakia and the Czech Republic it is 500–700 mm.

Symbol of Name of Country of Latitude and Longitude Genotype Genotype Cultivation organic farming 1.OF Unknown Poland 50°26' N, 16°39' E 2.OF 50°58' N, 16°81' E Unknown Poland 3.OF 50°58' N, 16°39' E Unknown Poland 49°58' N, 16°58' E 4.OF Mirel Czech 5.OF Slovakia 48°19' N, 17°43' E Silyb conventional farming 1.CF Silma Poland 51°17' N, 18°48' E 2.CFUnknown Poland 53°29' N, 18°45' E 3.CF Silma Slovakia 48°19' N, 18°08' E 4.CF 49°58' N, 16°58' E Mirel Czech 5.CF Silyb Czech 49°58′ N, 16°58′ E

Table 1. Symbols, names, and places of cultivation of milk thistle.

After cleaning the fruit and drying it in a dryer at 50 °C, samples were taken in order to test the content of total silymarin and the composition of flavonolignans (100 g), fat (200 g), and microbiological purity (200 g).

2.1. Determination of Silymarin and Oil Content

The content of silymarin, expressed as silybinin in milk thistle fruit, was tested in accordance with the monograph of the Pharmacopea [25], *Silybi mariani fructus* (01/2014:1860) by high-performance liquid chromatography (HPLC), using a liquid chromatograph equipped with a UV detector, Agilent HP 1260 in Poznańskie Zakłady Zielarskie Herbapol S.A (Poznań Herbal Plants).

Total oil content was determined gravimetrically using cyclohexane as solvent. The analyzes were performed in the accredited Eurofins Polska laboratory.

2.2. Dry Silymarin Extracts

To obtain dry silymarin extracts, methanol extracts from milk thistle fruit from organic and conventional farming, with determined silymarin content, were evaporated on a Heidolph vacuum rotary evaporator. The dry extract was dissolved in 3 mL of DMSO (dimethyl sulfoxide). This procedure was carried out to demonstrate that the antimicrobial activity of silymarin is not due to the presence of methanol residues.

2.3. Microbiological Quality Assessment

Microbiological quality assessment of the raw material was carried out in the accredited GBA POLSKA laboratory and was made based on Polish or European standards:

- 1. Total aerobic mesophilic count (TAMC), plate method, depth inoculation [26];
- 2. Total yeast and mold count (TYMC), plate method, surface inoculation [27];
- 3. Number of ß-glucuronidase-positive *Escherichia coli*, plate method, depth inoculation; [28];
- 4. Number of coliforms, plate method, depth inoculation [29];
- 5. Detection of *Listeria monocytogenes* in 25 g, breeding method supplemented with biochemical tests [30];
- 6. Detection of *Salmonella* spp. in 25 g, breeding method supplemented with biochemical tests [31].

The test results were referred to the guidelines contained in the Commission Regulation (EC) [32] as amended, the Regulation of the Minister of Health [33], and to the recommendations of the World Health Organization [34].

2.4. Evaluation of the Antimicrobial Properties of Silymarin

Twenty-four common etiological agents of skin infections (three of each species) were studied: *Staphylococcus aureus*, Methicillin-Resistant *S. aureus* (MRSA), *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

These were clinical strains obtained from the Department of Microbiology, Collegium Medicum, Nicolaus Copernicus University in Bydgoszcz. The microorganisms were grown on TSA medium for 24 h at 37 °C, then several single colonies of each strain were collected and suspensions with a density of 5×10^5 CFU/mL in BHI (Brain Heart Infusion) medium were prepared. The tests used silymarin solutions from organic (E) and conventional (K) production in DMSO (dimethylsulfoxide) at concentrations of 500 mg/mL. For tests checking their antimicrobial properties, they were diluted in BHI (at double concentration) to obtain solutions with concentrations of 250 mg/mL.

2.5. Minimum Inhibitory Concentration for Silymarin (MIC)

The minimum inhibitory concentrations of silymarin were determined by a serial broth microdilution method in BHI medium using U-bottom 96-well polystyrene microtiter plates (Profilab, 555.2.IS). A volume of 100 μ L of BHI broth was added to each well of a microplate and 100 μ L of the silymarin was used to conduct a two-fold serial dilution, giving concentrations of 250 to 1.8 mg/mL. Next, 10 μ L of the bacterial or yeast suspension was added to the corresponding rows. Wells containing only microorganisms in BHI were used as growth control, and wells with 100 μ L of DMSO in BHI with microorganisms were tested to exclude the antimicrobial effect of the solvent. As a sterility control, only growth medium without any microorganisms and silymarin was used. The cultures were incubated in a humid chamber at 37 °C for 24 h. Bacterial and fungal growth were evaluated according to turbidity. The tests were performed in triplicate. MIC was defined as the lowest concentration at which no growth was observed.

2.6. Minimum Bactericidal/Fungicidal Concentration for Silymarin (MBC/MFC)

The minimum bactericidal/fungicidal concentrations were determined based on broth dilution MIC tests by subculturing to selective agar media. Mannitol Salt Phenol Red Agar (Merck, 105404) was used to culture *S. aureus* and MRSA strains, Endo Agar (Merck, 1.04044) for *Escherichia coli*, Pseudomonas CN Selective Agar (Merck 107620) with Pseudomonas CN Selective Supplement (Merck, 1.07624) for *Pseudomonas aeruginosa*, and the Candida Chromogenic LAB-AGAR (Biomaxima, PS 665E) chromogenic medium for *Candida* spp. strains. The MBC/MFC is the lowest concentration of the agent that reduces populations by 99.9% of the tested bacteria/fungi.

2.7. Statistical Analysis

Statistical analysis was carried out for data on the content of silymarin, the percentage of individual components of silymarin (flavonolignans), and the oil content in milk thistle

fruits. The obtained results were statistically processed in terms of equality of variances and normality of distributions. Differences between the means were compared using the Student's *t*-test at the significance level of $\alpha = 0.10$. This level was adopted due to the small sample size.

The number of microorganisms on milk thistle fruits was given as an average for organic and conventional farming.

3. Result and Discussion

The average content of silymarin in milk thistle grown in organic and conventional farming systems was very similar (1.69 and 1.82%, respectively) (Table 2). This level seems relatively low compared to the values reported in other publications [8,12]. The difference results from the method of presenting the analysis results. This study was guided by the definition of the herbal raw material of *Silybi mariani fructus* given by the Pharmacopea [25], where silymarin is expressed as silybinin, and its content should not be lower than 1.5%.

Table 2. Content of silymarin (% DM), proportion of flavonolignans (%) and oil content (% DM) in milk thistle fruits cultivated in organic and conventional farming.

Symbol of Genotype	Silymarin Expressed as Silybinin	Silychristin Silydianin	Silybinin A Silybinin B	Isosilybinin A Isosilybinin B	Oil Content
1.OF	1.88	45.9	36.3	17.8	23.10
2.OF	1.28	48.7	27.6	23.6	24.80
3.OF	1.67	53.0	24.1	22.9	23.15
4.OF	2.32	27.2	61.2	11.6	22.94
5.OF	1.29	34.6	51.8	13.6	22.60
Mean	1.69 ^a	41.9 ^a	40.2 ^b	17.9 ^a	23.32 ^a
1.CF	1.67	29.8	58.0	12.2	23.54
2.CF	1.29	30.5	58.3	11.2	24.00
3.CF	1.66	27.8	59.8	12.4	24.96
4.CF	1.88	28.3	59.1	12.6	23.25
5.CF	2.61	29.7	56.7	13.6	23.79
Mean	1.82 ^a	29.2 ^b	58.4 ^a	12.4 ^b	23.91 ^a

The means marked vertically with the same letter do not differ significantly at the level of $\alpha = 0.10$.

In silymarin from organic milk thistle fruits, a significantly higher share of silychristin and silydianin and total isosilybinins was found, and a significantly lower share of total silydianins than in silymarin from conventional milk thistle fruits. In conventionally cultivated genotypes, the dominant flavonolignans were silybinin A and B (Table 2).

In the group of genotypes cultivated in the organic system, there were two cultivars with a dominant content of silybinin (5.OF-Mirel and 6.OF-Silyb). The three remaining genotypes were characterized by a dominant content of silychristin and silydianin; they also contained more isosilybinin A and B genotypes than other genotypes. The proportion of flavonolignans in the composition of silymarin is genetically determined. Two chemotypes of *S. marinum* are distinguished in the literature—chemotype A, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin composition is dominated by silybinin, and chemotype B, where the silymarin co

The cultivation system had no effect on the oil content of the milk thistle fruit (Table 2). The obtained results of the present study, in comparison with the results of other authors, also prove that the oil content in milk thistle fruit is slightly influenced by the genotype. In temperate climates, as a rule, the oil content in milk thistle fruit is lower than in fruit obtained in warmer climates [8,11,13,35].

The therapeutic effectiveness of herbal raw materials depends on the content of bioactive ingredients. Many factors affect their concentration, including: growing conditions, processing, or storage, as well as microbiological contamination that may reduce or even inactivate their therapeutic activity [36]. Primary contamination of raw materials is caused mainly by spoiling bacteria, yeasts, and molds originating from the soil, irrigation water, air, animal waste, as well as organic fertilizers (e.g., crop residues). The latter may additionally stimulate the growth of soil microorganisms and thus increase the degree of contamination of raw materials [37]. The soil intended for plant production, especially in organic farms, is often fertilized with manure or compost, which can be a breeding ground for pathogens such as *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and many others [38–40]. Secondary contamination of the raw material is associated with improper harvesting, lack of hygiene during drying and processing of harvested plants, storage, and transport, which applies to both organic and conventional farms [38,39].

Milk thistle fruits are the starting material for obtaining the extract which is the active substance in the medicinal product. In our study, organic fruit had significantly higher total microbial counts (TAMC) and yeasts and molds (TYMC) (Table 3). Bearing in mind the fulfillment of stringent standards for medicinal products, such a raw material may require additional decontamination processes before extraction [25].

Table 3. Number of microorganisms in samples of dried milk thistle fruit from organic and conventional farming.

Sample	TAMC [CFU·g ⁻¹]	TYMC [CFU·g ⁻¹]	Coliforms [CFU \cdot g ⁻¹]	Escherichia coli [CFU·g ⁻¹]	Salmonella spp. [in 25 g]	Listeria monocytogenes [in 25 g]
			organic farming			
1.OF	$2.5 imes10^6$	$1.0 imes 10^4$	$<1.0 \times 10^{1}$	$< 1.0 \times 10^{1}$	ND	ND
2.OF	$1.8 imes 10^7$	$9.4 imes 10^4$	$< 1.0 \times 10^{1}$	$< 1.0 \times 10^{1}$	ND	ND
3.OF	$9.5 imes10^6$	$6.0 imes 10^3$	$<1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	ND	ND
4.OF	$8.0 imes10^5$	7.1×10^3	$5.9 imes 10^3$	$<1.0 \times 10^{1}$	ND	ND
5.OF	$5.2 imes 10^6$	$8.8 imes10^4$	$<1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	ND	ND
Mean1-5.OF	$72 imes 10^5$	$4.1 imes 10^4$	$118.8 imes 10^1$	$<1.0 \times 10^{1}$	ND	ND
			conventional farmin	g		
1.CF	$7.4 imes10^5$	$1.9 imes10^4$	$< 1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	ND	ND
2.CF	$2.4 imes10^6$	$2.0 imes 10^4$	$<1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	ND	ND
3.CF	$2.2 imes 10^6$	$<1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	ND	ND
4.CF	$6.4 imes10^5$	$4.1 imes 10^2$	$<1.0 \times 10^{1}$	$<1.0 \times 10^{1}$	ND	ND
5.CF	$7.8 imes10^4$	$1.5 imes 10^2$	$1.5 imes10^4$	$<1.0 \times 10^{1}$	ND	ND
Mean1-5.CF	$1.2 imes 10^5$	$7.9 imes 10^3$	$300.8 imes 10^1$	$<1.0 \times 10^{1}$	ND	ND

TAMC—total microbial count; TYMC—total yeasts and molds count; CFU—colony-forming unit; ND—not detected.

Whole or ground milk thistle fruits are also treated as food products with high health benefits. For food products, regulations on food safety, including microbiological quality, are applied [32–34]. This may have been due to the properly conducted process of drying the fruit right after harvesting, which inhibited the development of vegetative forms [41]. It should also be mentioned that companies or farms with organic certificates have the option of decontaminating biologically contaminated plant material, e.g., by ozonation.

Herbal plants, like agricultural and horticultural plants, can be a habitat of a large number of bacteria and fungi, including potential organisms that cause the decomposition of the raw material. The microbiological quality is evidenced by the results of the quantitative analysis of indicator microorganisms, which reflect the general condition of the raw material or the environment in which it is processed. TAMC (total aerobic mesophilic count), coliforms, and *E. coli* are commonly determined [42]. In the present study, the number of TAMCs and the number of yeasts and molds in milk thistle fruits from the organic production system were almost six times higher than in products from conventional farming (Table 3). Fungi are considered as indicators used to assess the raw material quality, in particular in the context of its spoilage and forecasting the shelf life. In scientific publications, it is often emphasized that milk thistle fruits and dietary supplements produced on their basis are inhabited by fungi, including species that accumulate mycotoxins. Mycotoxins are a threat for both production systems [14,43,44]. Excluding the use of chemicals for plant protection in organic farming may lead to increased contamination of products with fungi that produce these toxic compounds. In the present study, milk thistle fruits were not tested for the presence of mycotoxinogenic species; however, such a situation cannot be ruled out [45].

Isolation of coliforms and *E. coli*, which occur in the digestive tracts of warm-blooded animals, from food samples indicates fecal contamination and the potential presence of pathogens. In the present study, coliforms were present in only one in five samples of fruit from both organic and conventional cultivation, while the raw material from the conventional system contained 2.5 times more bacteria of this group. It is believed that organic farms using natural fertilizers are more exposed to soil and raw material contamination with fecal bacteria, while we obtained the opposite relationship. The number of *Escherichia coli* did not exceed the recommended limits in any fruit sample, and no pathogens of *Salmonella* ssp. or *Listeria monocytogenes* were found.

The assessment of the antimicrobial properties of silymarin solutions in DMSO was tested against microorganisms that are common etiological agents of skin infections-Grampositive Staphylococcus aureus and MRSA, Gram-negative Escherichia coli and Pseudomonas aeruginosa, and yeasts of the genus Candida. The results are presented as the MIC and MBC/MFC of the compound in mg·mL⁻¹ (Table 4). Silymarin is a complex of substances containing flavonolignans, mainly silybinin or silydianin. Silybinin has hepatoprotective and antimicrobial activity, especially against Gram-positive bacteria, because it inhibits the synthesis of their RNA and protein [46]. In our research, however, it was shown that against Gram-positive bacteria S. aureus and MRSA, a silymarin extract with a high content of silvdianin and silvchristin (organic raw material) was more effective than a silvmarin extract with a predominance of the sum of silydianin (Tables 2 and 4). At the same time, silymarin did not inhibit the growth of Gram-negative bacteria—E. coli and P. aeruginosa, which is consistent with the reports of other researchers [46,47]. The microorganism most sensitive to its action was the methicillin-resistant strain of S. aureus (MRSA), for which the MIC/MBC was determined at the level of $15.6/31.25 \text{ mg} \cdot \text{mL}^{-1}$ for the product from the conventional system and $7.8/15.6 \text{ mg} \cdot \text{mL}^{-1}$ for the organic system. The antibacterial activity of silymarin against MRSA was also tested by Faezizadeh et al. [48] and the MIC of silymarin against the antibiotic-resistant strain was 500 mg/L, so it was much higher than in the present study. Such discrepancies may be the result of both different starting concentrations of silymarin and the use of bacteria that are less sensitive to the test compound.

Reports in the literature on the antifungal effect of silymarin confirm the effectiveness of the compound against both yeast and mold fungi [49]. Janeczko and Kochanowicz [50] studied the effect of silymarin against *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, determining the MIC to be in the range from 30 to 300 µg/mL. In our study, silymarin was less active, its MIC value ranged from 20–255 mg·mL⁻¹. One yeast species, *Candida glabrata*, was not sensitive to even the highest concentration of the preparation.

The results of the present study indicate that the silymarin extract from organic milk thistle crops had a more effective antimicrobial effect than that from the conventional system. This was most clearly seen for *S. aureus*, whose development was inhibited 4 times more strongly by the organic product. The organic extract also turned out to be a more

effective biocide against the MRSA strain. Two times higher anti-yeast activity of the organic extract was noticeable for *C. albicans* and *C. tropicalis*.

Microorganisms	Silymarin Extr Milk Th	ract from Organic istle Crops	Silymarin Extract from Conventional Milk Thistle Crops	
	MIC	MBC/MFC	MIC	MBC/MFC
Staphylococcus aureus	62.5	125.0	250.0	250.0
MRSA	7.8	15.6	15.6	31.25
Escherichia coli	х	х	х	х
Pseudomonas aeruginosa	х	х	х	х
Candida albicans	125.0	125.0	125.0	250.0
Candida krusei	250.0	250.0	125.0	250.0
Candida glabrata	х	х	х	х
Candida tropicalis	125.0	125.0	250.0	250.0

Table 4. Antimicrobial activity $[mg \cdot mL^{-1}]$ of silymarin extract from organic and conventional farming.

MIC—Minimum inhibitory concentration; MBC/MFC—Minimum bactericidal/fungicidal concentration; x—no action.

Differences in the effectiveness of antimicrobial activity may result from the composition of silymarin. Among the raw materials from organic farming, three genotypes represented chemotype B with a predominance of silybinin and silychristin, while in the group of raw materials from conventional farming, all genotypes represented chemotype A with silybinin as the basic component. There are mentions in the literature of silydianin having a different action than silybinin [16]. According to Zielińska-Przyjemska and Wiktorowicz [51], silydianin is an effective inhibitor of free radicals production and release and this can partly explain the therapeutic use of silydianin as an anti-inflammatory drug. However, this issue requires further research. If the above hypothesis is confirmed, it will be justified to breed and register cultivars with a high content of silydianin in silymarin, adapted to the conditions of a temperate climate.

The present study brought the following new knowledge on the quality of milk thistle fruits:

- Herbal raw material from organic farming obtained in Central Europe may be characterized by a different composition of silymarin, and a high content of silychristin and silydianin in the composition of silymarin;
- Silymarin, especially genotypes with a high total content of silychristin and silydianin, is a promising raw material for production of ointments for skin infections;
- Despite the fact that milk thistle fruits from organic farming are more microbiologically contaminated than those from conventional crops, they meet the standards for food products, especially since *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* have not been found on them.

4. Conclusions

Milk thistle, due to its versatile use, is an interesting plant for organic farms. They can primarily offer food products such as whole or ground fruit or pressed oil. Raw material from some organic crops, compared to raw material from conventional crops, may differ in the composition of silymarin—a higher proportion of the sum of silydianin and silychristin than the sum of silybinins. Drying and storage processes carried out properly allow a raw material to be obtained in which the level of microbiological contamination (yeast, molds) is within the standards provided for food products. Importantly, this organic raw material was also found to be free of *Escherichia coli, Salmonella* spp., and *Listeria monocytogenes*. The antimicrobial properties of silymarin, especially the one rich in silydianin and silychristin, give an opportunity to expand the offer with certified ointments and creams used in cases of skin infection and damage. However, this still requires extended and in-depth research.

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