

Article

Whether supercritical extracts from the aerial parts of *Helianthus salicifolius* and *Helianthus tuberosus* may be regarded as a potential raw materials for non-bioenergy purposes?

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Abstract: The extracts from the aerial parts of *Helianthus salicifolius* A. Dietr and *Helianthus tuberosus* L. collected in June were obtained using carbon dioxide supercritical fluid extraction with water as co-solvent. The antimicrobial activity *in vitro* of these extracts were determined against the reference species of Gram-positive and Gram-negative bacteria as well as fungi, representing by the yeast species of *Candida* spp. The following parameters were estimated: minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC). Both extracts were found to possess antimicrobial activity with MIC = 0.62-5 mg mL⁻¹ for bacteria and MIC = 5-10 mg mL⁻¹ for yeasts, showing bactericidal (MBC/MIC = 2-4) or fungicidal effect (MFC/MIC = 1-2). The highest activity was observed against *S. aureus* ATCC 29213 (MIC = 0.62 mg mL⁻¹ for *H. salicifolius* extract; MIC = 2.5 mg mL⁻¹ for *H. tuberosus* extract). Bactericidal effect of both extracts against *S. aureus* ATCC 29213 was confirmed by time-kill assay. Higher antioxidant activity was found for *H. tuberosus* extract (EC₅₀ = 0.332 mg mL⁻¹) as compared to that of *H. salicifolius* (EC₅₀ = 0.609 mg mL⁻¹). The total polyphenol content (TPC) expressed as gallic acid equivalents (GAE) was 13.75 ± 0.50 mg GAE (g of *H. salicifolius* extract)⁻¹ and 33.06 ± 0.80 mg GAE (g of *H. tuberosus* extract)⁻¹. There was a correlation between the antioxidant potential of both extracts and TPC but not between antistaphylococcal activity and TPC. The obtained data suggest potential application of these extracts as the natural preparations with the biocidal activity, including those with antistaphylococcal activity. Besides, both extracts may be regarded as potential natural conservants in cosmetics as well as natural preservatives in food.

Keywords: willow-leaf sunflower; Jerusalem artichoke; supercritical extraction; water as co-solvent; antimicrobial activity; biocidal effect

1. Introduction

Perennial herbaceous crops, including *Helianthus tuberosus* L. (named also as Jerusalem artichoke or topinambur) and *Helianthus salicifolius* A. Dietr. (willow-leaf sunflower) belong to the group of plants of potentially high importance for energy use [1,2]. This is due to high biomass production and limited cultivation requirements. It should be added that these species are resistant to frost and possible infestation by diseases and pests. However, when harvesting the aerial parts biomass of these species, there may be periodic lodging problems, which may make harvesting difficult, but this occurs mainly at the end of the vegetation period [2]. The biomass of these species can be a raw material for the production of biogas, liquid biofuels and solid biofuels [3-6]. However, it should be emphasized that the energetic use of biomass of these species is one of the simplest and least effective methods of its management. Therefore, new possibilities of using the biomass of these species should be searched for in order to obtain bioactive substances from it and their further use for the production of high-value bioproducts.

It is well-known that plants are valuable and rich source of a wide range of secondary metabolites, possessing multidirectional biological activity [7]. It was revealed that *H. tuberosus* exerted antioxidant, anticancer, antidiabetic and α -glucosidase inhibitory activity, as well as it produced inulin which used as functional food and possessed many medical benefits [8]. Moreover, there are a few literature data reporting antimicrobial activity of *H. tuberosus* but only against several fungal phytopathogens such as *Rhizoctonia solani*, *Gibberella zeae*, *Alternaria solani*, *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Phytophthora capsici* Leonian [9-11]. However, no data concerning antimicrobial (antifungal) properties of *H. salicifolius* are available.

The aim of this study was to determine the antimicrobial properties of the extracts obtained from the aerial parts of *H. salicifolius* and *H. tuberosus* using carbon dioxide supercritical fluid extraction with water as co-solvent. These extracts were assayed for their activity against the Gram-positive and Gram-negative bacteria as well as fungi (yeasts), being the component of human skin, oral and gut microbiota, while under predisposing conditions – human pathogens [12-14]. The antimicrobial activity of these extracts was analyzed in a correlation with their total polyphenol content (TPC) and antioxidant properties.

2. Materials and Methods

2.1. Plant Material

The green aerial parts biomass of *H. salicifolius* and *H. tuberosus* were collected in 24 June 2019 from the experimental plantation owned by University of Warmia and Mazury in Olsztyn (Figure 1). These were plants from the beginning of the growing season (April-June) obtained from nine-year-old stools. The plants were harvested with a lawn trimmer and weighed on an electronic scales. During the harvest of plants, biomass samples were obtained in order to determine its moisture content. On the basis of the yield of fresh biomass and its moisture content during harvest, the yield of dry biomass was calculated and expressed in Mg ha⁻¹. After harvesting, the biomass of *H. salicifolius* and *H. tuberosus* was transported to the drying plant. Biomass was dried at 40° C to a moisture level below 10%. After drying it was ground using a mill with 6 mm mesh sieves. After grinding, the biomass was packed in bags and transported to the supercritical extraction plant.



Figure 1. (a) *H. salicifolius* and (b) *H. tuberosus* plants during the harvest period 24 June 2019 (photo: M.J.S.).

2.2. Extraction Method

The ground material was extracted with supercritical carbon dioxide with the addition of water as co-solvent in the amount of 1 wt.%. The supercritical fluid extraction was performed on a pilot plant produced by Natex, Austria, with two extractors of 40 dm³ each, working under the pressure of up to 1000 bar and temperature up to 90°C. Each raw material (5 kg for *H. salicifolius* and approx. 2 kg for *H. tuberosus*) was extracted with co-solvent under parameters, which were set as follows: temperature at 40°C and pressure at 330 bar. The extract obtained was in the form of an aqueous mixture. The water was evaporated using a Buchi R-220SE vacuum evaporator. The extraction yield expressed in % was determined for the dried extract in relation to the raw material as the ratio of the amount of dried extract to the mass of raw material. These supercritical extracts were named further as CO₂+H₂O extracts.

2.3. Determination of Total Polyphenol Content (TPC)

The total phenolic content in CO₂+H₂O extracts from *H. salicifolius* and *H. tuberosus* was determined spectrophotometrically by a modified method previously described by Clarke et al. [15] and Nickavar and Esbati [16]. A 20 µL of extract dissolved in DMSO (conc. 10 mg/mL) and 100 µL of freshly prepared Folin-Ciocalteu reagent (diluted 1/10 with redistilled water) were added to the wells of a 96-well plate. After 5 minutes, 100 µL of a 7.5% Na₂CO₃ solution was added. The plates with the mixtures were incubated for 60 min. at room temperature, then the absorbance was measured using an EPOCH spectrophotometer (Biotek, USA, Software ver. 3.08.01) at a wavelength of 760 nm. The same method was used to establish a calibration curve for the standard gallic acid in the concentration ranges 7.5-120.0 µg mL⁻¹ ($y = 0.054x + 0.029$, $R^2 = 0.996$). The analysis was performed in triplicate using DMSO as the blank. The content of total phenolic content expressed in equivalents as mg GAE/g of extract was calculated according to the formula [17].

2.4. Determination of Antibacterial and Antifungal Activity

The assay of antibacterial and antifungal activity of CO₂+H₂O extracts from *H. salicifolius* and *H. tuberosus* was performed by broth microdilution method according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) recommendations [18]. The following reference strains were used in the study: *Staphylococcus aureus* ATCC 29213 (representative of Gram-positive bacteria), *Escherichia coli* ATCC 25922 (representative of Gram-negative bacteria), *Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 90030 (representatives of fungi belonging to yeasts). All the used microbial strains were first subcultured on Mueller-Hinton Agar (MHA for bacteria) or Mueller-Hinton Agar with 2% glucose (MHA+2% glucose for fungi) and incubated at 35°C for 24 h. Microbial colonies were collected and suspended in sterile physiological saline to obtain inoculum of 0.5 McFarland standard, corresponding to 1.5×10^8 CFU (colony forming units) mL⁻¹ for bacteria and 5×10^6 CFU mL⁻¹ for fungi. The CO₂+H₂O extracts were dissolved in DMSO to obtain the final concentration 100 mg/mL. The two-fold dilutions of the extracts in Mueller-Hinton Broth (MHB for

bacteria) or by Mueller-Hinton Broth with 2% glucose (MHB+2% glucose for fungi) were prepared in 96-well polystyrene plates. The final concentrations of the extracts ranged from 40 to 0.155 mg mL⁻¹. Next 2 µl of each bacterial or fungal inoculum was added to each well containing 200 µl of the serial dilution of the extracts in the appropriate culture medium. After incubation at 35°C for 24 h, the MIC (minimum inhibitory concentration) was assessed spectrophotometrically as the lowest concentration of the extract showing complete bacterial or fungal growth inhibition. Appropriate DMSO, growth and sterile controls were carried out. Besides, vancomycin (0.03-10 µg mL⁻¹), ciprofloxacin (range of 0.007-10 µg mL⁻¹) and fluconazole (0.03-10 µg mL⁻¹) were included as the reference antimicrobial substances active against Gram-positive bacteria, Gram-negative bacteria and yeasts. The MBC (Minimal Bactericidal Concentration) or MFC (Minimal Fungicidal Concentration) was determined by removing 20 µl of the bacterial or fungal culture using for MIC determinations from each well and spotting onto appropriate agar medium. The plates were incubated at 35°C for 24 h. The lowest extracts concentrations with no visible bacterial or fungal growth was assessed as MBC or MFC, respectively. The experiments were performed in triplicate.

2.5. Determination of Antioxidant Activity

The antioxidant activity of CO₂+H₂O extracts from *H. salicifolius* and *H. tuberosus* extracts was determined using the method described by Gai et al. [19] with modifications. Briefly, a starting solution was prepared by dissolving 10 mg of extract in 1 mL of DMSO solution. Then a series of dilutions were prepared in the same solvent at a concentration of 0.16-10 mg mL⁻¹. A 0.05 mL of each concentration was mixed with 0.15 mL of DPPH methanol solution (0.078 mg mL⁻¹). The 96-well plate with the mixtures was incubated in the dark for 30 min in room temperature. Absorbance was measured at 515 nm (Biotek, Epoch, Software Version 3.08.01). The extract concentration needed to capture 50% of the initial DPPH (EC₅₀) was determined automatically using 4 parameter logistic regression (4LP) from the plate reader software Gen5. The experiments were performed in triplicate.

3. Results

The height of the nearly three-month-old plants of both species was 1.1 m (Table 1). *H. salicifolius* produced slightly thicker shoots and therefore the yield of fresh biomass for this species was higher and amounted to 12.9 Mg ha⁻¹. Moisture of *H. salicifolius* biomass was 79% and was almost two percentage points lower compared to that of *H. tuberosus*. Therefore, the dry matter yield of *H. salicifolius* harvested at the end of June was 2.7 Mg ha⁻¹ and was higher by 0.5 Mg ha⁻¹ compared to the yield of *H. tuberosus*.

Table 1. Biometric features, moisture content and yield of fresh and dry biomass of *H. salicifolius* and *H. tuberosus*.

Species	Plant height (m)	Shoot diameter (mm)	Fresh biomass yield (Mg ha ⁻¹)	Moisture content (%)	Dry biomass yield (Mg ha ⁻¹)
<i>H. salicifolius</i>	1.1±0.9	7.0±1.0	12.9±2.2	79.1±0.4	2.7±0.4
<i>H. tuberosus</i>	1.1±0.4	5.9±0.6	11.6±3.3	81.0±1.1	2.2±0.8

Mean values ±standard deviation were presented.

The results presented in Table 2 showed that the extraction efficiency of *H. salicifolius* (4.97%) was much higher than that of *H. tuberosus*. Due to the fact that the yield of *H. salicifolius* aerial parts biomass was also higher, the amount of extract that could be obtained from the cultivation of this species was approximately 134 kg ha⁻¹. On the other hand, the production potential of *H. tuberosus* extract was almost 20 times lower.

Table 2. Extraction efficiency and extract potential yield of *H. salicifolius* and *H. tuberosus* from dry biomass under supercritical conditions with the participation of water as a co-solvent (CO₂+H₂O extracts).

Plant material	Extraction efficiency (%)	Extract potential yield (kg ha ⁻¹)
<i>H. salicifolius</i>	4.97	134.19
<i>H. tuberosus</i>	0.31	6.82

As shown in Table 3 and 4, the CO₂+H₂O extracts obtained from *H. salicifolius* and *H. tuberosus* showed differential activity against bacteria (MIC = 0.62-5 mg mL⁻¹) and yeasts (MIC = 5-10 mg mL⁻¹). The highest activity of both extracts was observed against *S. aureus* ATCC 29213 with MIC = 0.62 mg mL⁻¹ for *H. salicifolius* extract and MIC = 2.5 mg mL⁻¹ for *H. tuberosus* extract. Besides, MIC for the reference antimicrobial substances were as the following: MIC of vancomycin for *S. aureus* ATCC 29213 was 1 µg mL⁻¹, MIC of ciprofloxacin for *E. coli* ATCC 25922 was 0.015 µg mL⁻¹ and MIC of fluconazole for *C. albicans* ATCC was 1 µg mL⁻¹. As presented in Table 3 and 4, both CO₂+H₂O extracts possessed bactericidal (MBC/MIC = 1-4) and fungicidal effect (MFC/MIC = 1-2). It is generally accepted that antimicrobials are usually regarded as bactericidal or fungicidal if the MBC/MIC or MFC/MIC ratio is ≤ 4 [20].

Table 3. Antimicrobial activity of supercritical extract obtained from *H. salicifolius* with water as a co-solvent (CO₂+H₂O extract).

Antibacterial activity			
Strain	MIC* (mg mL ⁻¹)	MBC* (mg mL ⁻¹)	MBC/MIC*
<i>Staphylococcus aureus</i>	0.62	2.5	4
ATCC 29213	(0.31;0.62;0.62)	(1.25;2.5;2.5)	(4;4;4)
<i>Escherichia coli</i>	5	10	2
ATCC 25922	(5;5;5)	(10;10;10)	(2;2;2)
Antifungal activity			
Strain	MIC (mg mL ⁻¹)	MFC (mg mL ⁻¹)	MFC/MIC
<i>Candida albicans</i>	5	10	2
ATCC 10231	(5;5;5)	(10;10;10)	(2;2;2)
<i>Candida glabrata</i>	10	10	1
ATCC 90030	(10;10;10)	(10;10;10)	(1;1;1)

*The representative (modal) data are presented. Values in parentheses were determined in the separate experiments.

Table 4. Antimicrobial activity of supercritical extract obtained from *H. tuberosus* with water as a co-solvent (CO₂+H₂O extract).

Antibacterial activity			
Strain	MIC* [mg mL ⁻¹]	MBC* [mg mL ⁻¹]	MBC/MIC*
<i>Staphylococcus aureus</i>	2.5	5	2
ATCC 29213	(1.25;2.5;2.5)	(2.5;5;5)	(2;2;2)
<i>Escherichia coli</i>	5	5	1
ATCC 25922	(5;5;5)	(5;5;5)	(1;1;1)
Antifungal activity			
Strain	MIC [mg mL ⁻¹]	MFC [mg mL ⁻¹]	MFC/MIC
<i>Candida albicans</i>	5	10	2
ATCC 10231	(5;5;10)	(10;10;10)	(2;2;1)
<i>Candida glabrata</i>	10	20	2
ATCC 90030	(5;10;10)	(10;20;20)	(2;2;2)

See Legend of Table 3.

Time-kill assays were performed exposing *S. aureus* ATCC 29213 to various concentrations of the CO₂+H₂O extracts obtained from *H. salicifolius* and *H. tuberosus* in order to confirm their bactericidal activity. It is assumed that bactericidal effect is defined as greater than 3 log₁₀-fold decrease in CFU mL⁻¹ in the presence of antimicrobials as compared to the initial inoculum [21]. As presented in Figure 2, bacterial killing by both extracts was found to be a concentration-dependent process; some biocidal effect occurred even at sub-inhibitory concentrations of both extracts, that was 0.1 mg mL⁻¹ for *H. salicifolius* extract and 1 mg mL⁻¹ for *H. tuberosus* extract. Moreover, *H. salicifolius* extract was more active than that of *H. tuberosus*.

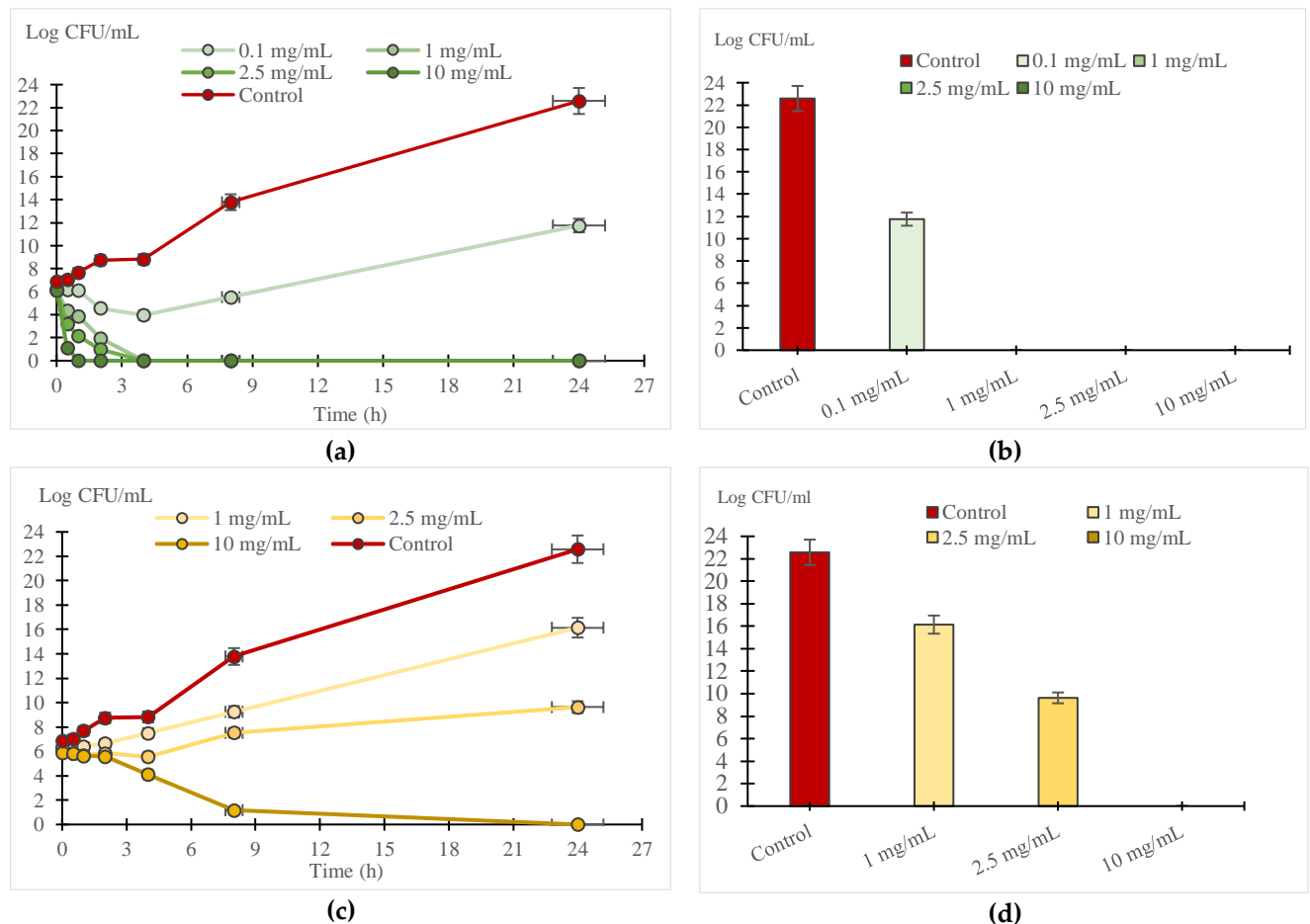


Figure 2. Time-kill curves for *S. aureus* ATCC 29213 at various concentrations of supercritical extracts obtained from: (a) *H. salicifolius* and (c) *H. tuberosus* with water as a co-solvent ($\text{CO}_2+\text{H}_2\text{O}$ extracts). Bacterial population density after 24 h exposure to various concentrations of the $\text{CO}_2+\text{H}_2\text{O}$ extracts obtained from: (b) *H. salicifolius* and (d) *H. tuberosus*. Mean values \pm standard deviation were presented.

As presented in Figure 3, the $\text{CO}_2+\text{H}_2\text{O}$ extracts obtained from *H. salicifolius* and *H. tuberosus* differed in the total polyphenol content (TPC) expressed as gallic acid equivalents (GAE). It was 13.75 ± 0.50 mg GAE (g of *H. salicifolius* extract⁻¹) and 33.06 ± 0.80 mg GAE (g of *H. tuberosus* extract⁻¹). Both extracts showed different antioxidant activity. *H. tuberosus* extract exhibited almost two-fold higher activity ($\text{EC}_{50} = 0.332 \pm 0.05$ mg mL⁻¹) as compared to that of *H. salicifolius* ($\text{EC}_{50} = 0.609 \pm 0.29$ mg mL⁻¹). There was a correlation between the antioxidant potential of both extracts and TPC. It should be noted that there was no correlation between antistaphylococcal activity of both extracts (Figure 2) and the TPC (Figure 3). *H. salicifolius* extract showed four-fold higher activity against *S. aureus* ATCC 29213 ($\text{MIC} = 0.62$ mg mL⁻¹) than that *H. tuberosus* extract ($\text{MIC} = 2.5$ mg mL⁻¹).

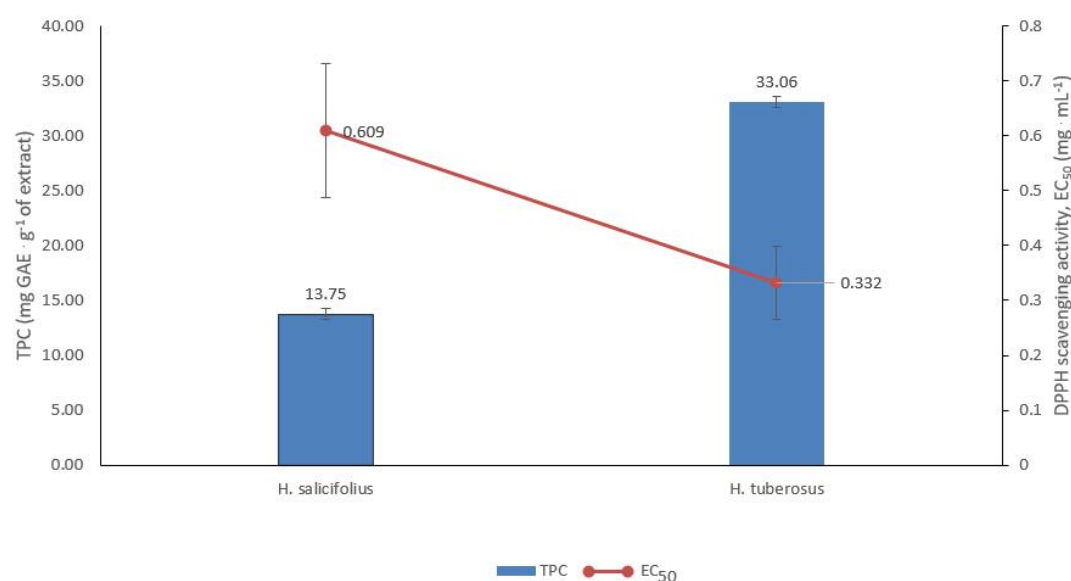


Figure 3. The total polyphenol content (TPC) in supercritical extracts obtained from *H. salicifolius* and *H. tuberosus* with water as a co-solvent (CO₂+H₂O extracts) together with their antioxidant activity. Mean values ± standard deviation were presented.

4. Discussion

Recently, much attention has been paid to various plants as a source of alternative antimicrobial compounds and strategies [7]. According to the literature data [9-11], the extracts from *H. tuberosus* leaves might be a promising source of natural fungicides active against several phytopathogens, among them caffeoylquinic acid derivatives. Liu et al. [9] found that the inhibitory effects of aqueous extracts were significantly less than those of extracts of organic solvents, *i.e.* petroleum ether, ethyl ether and ethyl acetate. Phytochemical analysis of *H. tuberosus* showed that it contained coumarins, unsaturated fatty acids, polyacetylenic derivatives, phenolic acids, flavonoids and sesquiterpene lactones [8].

Data presented in this paper showed that CO₂+H₂O extracts obtained not only from *H. tuberosus* but also from *H. salicifolius* showed antimicrobial potential, including activity against bacteria (*S. aureus*, *E. coli*) as well as yeasts (*C. albicans*, *C. glabrata*). Both extracts exerted bactericidal and fungicidal effect. It should be noted that the above microbial species present within human skin, oral and gut microbiota may be regarded as commensals or pathogens, depending on the host-microbe interactions [12-14]. Moreover, these microorganisms may be a cause of cosmetics or food contamination, hence the need to protect these products by substances with antimicrobial activity – conservants or preservatives, respectively [22,23].

Noteworthy is antistaphylococcal activity of CO₂+H₂O extracts obtained from *H. salicifolius* and from *H. tuberosus*. *S. aureus* is known to be an important pathogen related to skin and soft tissue as well as to food-borne infections [24]. In this paper we found higher antistaphylococcal effect of *H. salicifolius* CO₂+H₂O extract as compared to that of *H. tuberosus* CO₂+H₂O extract. In contrast, it was found that TPC was higher in *H. tuberosus* CO₂+H₂O extract in comparison to that in *H. salicifolius* CO₂+H₂O extract. Showkat et al. [25] found that TPC in *H. tuberosus* was dependent on the plant organ. They determined higher TPC in leaves than in flower, tuber and stem. It should be noted that polyphenols have been recognized as one of the largest and most widespread group of plant secondary metabolites, responsible for both antimicrobial and antioxidant activity [26]. Data presented in this paper suggest that the antistaphylococcal activity of both CO₂+H₂O extracts, especially that from *H. salicifolius*, may be due to the content of other plant secondary metabolites such as sesquiterpene lactones [27]. These compounds can be regarded as one of the most prevalent

and biologically significant classes of plant secondary metabolites, including plants from Asteraceae family, e.g. *H. tuberosus* [28] sesquiterpene lactones have been assumed to be potent antimicrobials. Some of them are considered to be antioxidants [29,30].

The antimicrobial properties of plant-derived products are generally accompanied by the confirmed antioxidant capacity [26-30]. In this paper we found higher antioxidant effect of *H. tuberosus* CO₂+H₂O extract together with its higher TPC as compared to those of *H. salicifolius* CO₂+H₂O extract. These data suggest that the antioxidant properties of CO₂+H₂O extracts studied may be related to polyphenols, which is in agreement with the literature data [31]. The TPC and radical scavenging (antioxidant) activity of *H. tuberosus* leaves were investigated by Yuan et al. [32]. They suggested, similarly as Showkat et al. [25], that the leaves of this plant could be a potential source of natural antioxidants.

5. Conclusions

The supercritical extracts from *H. salicifolius* and *H. tuberosus* aerial parts with water as a co-solvent appeared to be a promising source of natural compounds with antibacterial and antifungal activity, showing biocidal effects. Moreover, these extracts may be regarded as natural potential antioxidants as well as natural conservants in cosmetics or natural preservatives in food. However, further studies are needed to confirm the obtained results and to identify specific applications of supercritical extracts from biomass of these two plant species.

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