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Could Supercritical Extracts from the Aerial Parts of *Helianthus salicifolius* A. Dietr. and *Helianthus tuberosus* L. Be Regarded as Potential Raw Materials for Biocidal Purposes?

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Abstract: Extracts from the June collection of aerial parts of *Helianthus salicifolius* A. Dietr and *Helianthus tuberosus* L. were obtained using carbon dioxide supercritical fluid extraction with water as co-solvent. The antimicrobial effect in vitro of these extracts was then determined against reference species of bacteria, as well as against fungi (represented by *Candida* spp.). 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, and both extracts demonstrated suitable bactericidal and fungicidal effect. 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) as confirmed by time–kill assay. Higher antioxidant activity was found for *H. tuberosus* extract (EC_{50} = 0.332 mg mL^{-1}) as compared to that of *H. salicifolius* (EC_{50} = 0.609 mg mL^{-1}). The total polyphenol content (TPC) expressed as gallic acid equivalents (GAE) was 13.75 ± 0.50 mg GAE g^{-1} of *H. salicifolius* extract and 33.06 ± 0.80 mg GAE g^{-1} of *H. tuberosus* extract. There was a relationship between the antioxidant potential of both extracts and TPC, but not between antistaphylococcal activity and TPC. The ATIR–FTIR spectra of both extracts showed similar main vibrations of the functional groups typical for phytoconstituents possessing bioactivity. The obtained data suggest potential application of these extracts as natural antioxidants and preparations with biocidal activity. Additionally, 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

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1. Introduction

Perennial herbaceous crops, including *Helianthus tuberosus* L. (also called 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]. While the biomass of these species can be a raw material for the production of biogas, liquid biofuels, and solid biofuels [3–6], it should be emphasized that the energetic use of biomass of these species maybe of the least value regarding their production purposes.

Recently, much attention has been paid to various plants as a source of alternative antimicrobial compounds and strategies. It is well-known that plants are valuable and rich sources of a wide range of secondary metabolites that possess multidirectional biological activity [7]. Studies suggest that *H. tuberosus* exerts antioxidant, anticancer, antidiabetic, and α-glucosidase inhibitory activity and produces inulin. Indeed, studies have noted the antimicrobial activity of *H. tuberosus*, e.g., against several fungal phytopathogens such as *Rhizoctonia solani, Gibberella zeae, Alternaria solani*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Phytophthora capsici* Leonian [8–10]. However, no data concerning antimicrobial (antifungal) properties of *H. salicifolius* are available. The possibility, therefore, exists that Jerusalem artichoke and willow-leaf sunflower can be used as functional food with many medical benefits [11,12]. Thus, new possibilities of using the biomass of these species should be searched for in order to obtain bioactive substances from them and ascertain their further application in the production of high-value bioproducts.

The aim of this study was to determine the antimicrobial properties of extracts obtained from the aerial parts of *H. salicifolius* and *H. tuberosus* using carbon dioxide supercritical fluid extraction with water as co-solvent. The biomass for the extraction purposes was collected at the end of June. The extracts were assayed for their activity together with the mode of action (bactericidal/fungicidal vs. bacteriostatic/fungistatic) against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and against fungi (yeasts from *Candida* spp.)—these being the component of human skin, oral, and gut microbiota, which under predisposing conditions can be considered human pathogens [13–15]. The antimicrobial activity of these extracts was analyzed in a correlation with their total polyphenol content (TPC) and antioxidant properties. Attenuated total reflection–Fourier transform infrared (ATR–FTIR) spectra analyses of the extracts were also performed in order to obtain preliminary data on the presence of functional groups characteristic for bioactive phytoconstituents.

2. Materials and Methods

2.1. Plant Material

The green aerial parts biomass of *H. salicifolius* and *H. tuberosus* were collected on June 24, 2019, from the experimental plantation owned by the University of Warmia and Mazury in Olsztyn (Figure 1). This was biomass from the beginning of the growing season (April–June) obtained from nine-year-old stools. The plot size was 20 m^2. The plants were harvested with a lawn trimmer and weighed on an electronic scales. During the harvest, 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}. The number of replications was three. After harvesting, the biomass of *H. salicifolius* and *H. tuberosus* was transported to the drying plant. This biomass was then dried at 40 °C to a moisture level below 10%. After drying, it was ground using a mill with 6 mm mesh sieves. Subsequently, the biomass was packed in bags and transported to the supercritical extraction plant.

(**a**) (**b**)

Figure 1. (**a**) *H. salicifolius* and (**b**) *H. tuberosus* plants during the harvest period June 24, 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 dm^3 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 that 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 CO_2 + H_2O extracts.

2.3. Determination of Total Polyphenol Content (TPC)

The total phenolic content in CO_2 + H_2O extracts from *H. salicifolius* and *H. tuberosus* was determined spectrophotometrically by a modified method previously described by Clarke et al. [16] and Nickavar and Esbati [17]. A 20 µL of extract dissolved in DMSO (conc. 10 mg mL^{-1}) 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 min, 100 µL of a 7.5% Na_2CO_3 solution was added. The plates with the mixtures were incubated for 60 min. at room temperature; the absorbance was then 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} ($y = 0.054\,x + 0.029$, $R^2 = 0.996$). 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 described elsewhere [18].

2.4. Determination of Antibacterial and Antifungal Activity

The assay of antibacterial and antifungal activity of CO_2 + H_2O extracts from *H. salicifolius* and *H. tuberosus* was performed by the broth microdilution method according to EUCAST (the European Committee on Antimicrobial Susceptibility Testing) recommendations [19]. 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 yeast fungi). 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 CO_2 + H_2O extracts were dissolved in DMSO to obtain the final concentration 100 mg mL^{-1}.

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. Vancomycin (range of 0.03–10 µg mL^{-1}), ciprofloxacin (range of 0.007–10 µg mL^{-1}), and fluconazole (range of 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 (minimum bactericidal concentration) or MFC (minimum fungicidal concentration) were determined by removing 20 µL of the bacterial or fungal culture used for MIC determinations from each well and spotting this 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 were assessed as MBC or MFC, respectively. The experiments were performed in triplicate. Of the three MIC, MBC, and MFC values, the most common representative value, i.e., mode was presented.

2.5. Determination of Antioxidant Activity

The antioxidant activity of CO_2 + H_2O extracts from *H. salicifolius* and *H. tuberosus* extracts was determined using the method described by Gai et al. [20] with modifications. Briefly, a starting solution was prepared by dissolving 10 mg of extract in 1 mL of DMSO solution. A series of dilutions were then prepared in the same solvent at a concentration of 0.16–10 mg mL^{-1}. Subsequently, 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 (EC_{50}) was determined automatically using four parameter logistic regression (4LP) from the plate reader software Gen5. The experiments were performed in triplicate.

2.6. Attenuated Total Reflection-Fourier Transform Infrared (ATR–FTIR) Spectra Analysis

ATR–FTIR spectra of CO_2 + H_2O extracts obtained from *H. salicifolius* and *H. tuberosus* were recorded on a Bruker Tensor 27 FTIR spectrometer (Bruker Optic GmbH, Aetlingen, Germany) equipped with single-bounce diamond ATR (Platinum ATR, Bruker Optic GmbH, Aetlingen, Germany). The spectrometer was controlled with the software OPUS 6.5 (Bruker Optic). The scan number of the spectra was 16 recorded at 4 cm^{-1} resolution in the wavenumber range from 4000 to 400 cm^{-1}. A small amount of each extract (5 mg) was placed on the ATR surface that was cleaned using ethanol to eliminate any contamination by the previous sample. A new background was recorded between each replicate, and the scans were run in triplicates.

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}) **
H. salicifolius	1.1 ± 0.9	7.0 ± 1.0	12.9 ± 2.2	79.1 ± 0.4	2.7 ± 0.4
H. tuberosus	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; * n = 30; ** n = 3.

The results presented in Table 2 show 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 (CO_2 + H_2O extracts).

Plant Material	Extraction Efficiency (%)	Extract Potential Yield (kg ha^{-1})
H. salicifolius	4.97	134.19
H. tuberosus	0.31	6.82

As revealed in Table 3, the CO_2 + H_2O 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. 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*, and ATCC was 1 µg mL^{-1}. As presented in Table 3, both 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 [21].

Table 3. Antimicrobial activity of supercritical extracts obtained from *H. salicifolius* and *H. tuberosus* with water as a co-solvent (CO_2 + H_2O extract).

Microorganisms	Extracts					
	H. salicifolius			*H. tuberosus*		
Bacterial Strains	**MIC [mg mL^{-1}]**	**MBC [mg mL^{-1}]**	**MBC/ MIC**	**MIC [mg mL^{-1}]**	**MBC [mg mL^{-1}]**	**MBC/ MIC**
Staphylococcus aureus ATCC 29213	0.62	2.5	4	2.5	5	2
Escherichia coli ATCC 25922	5	10	2	5	5	1
Fungal (Yeasts) Strains	**MIC [mg mL^{-1}]**	**MFC [mg mL^{-1}]**	**MFC/ MIC**	**MIC [mg mL^{-1}]**	**MFC [mg mL^{-1}]**	**MFC/ MIC**
Candida albicans ATCC 10231	5	10	2	5	10	2
Candida glabrata ATCC 90030	10	10	1	10	20	2

The representative data (mode) are presented.

Time–kill assays were performed exposing *S. aureus* ATCC 29213 to various concentrations of the CO_2 + H_2O extracts obtained from *H. salicifolius* and *H. tuberosus* in order to confirm their bactericidal activity. *S. aureus* ATCC 29213 was chosen for this experiment due to its higher sensitivity to both extracts in comparison to *E. coli* ATCC 25922 and the yeast species. Moreover, it is a common human pathogen that causes a wide range of clinical infections. It is assumed that bactericidal effect is defined as greater than 3 $\log_{10}$-fold decrease in CFU mL^{-1} in the presence of antimicrobials as compared to the initial inoculum [22]. 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*.

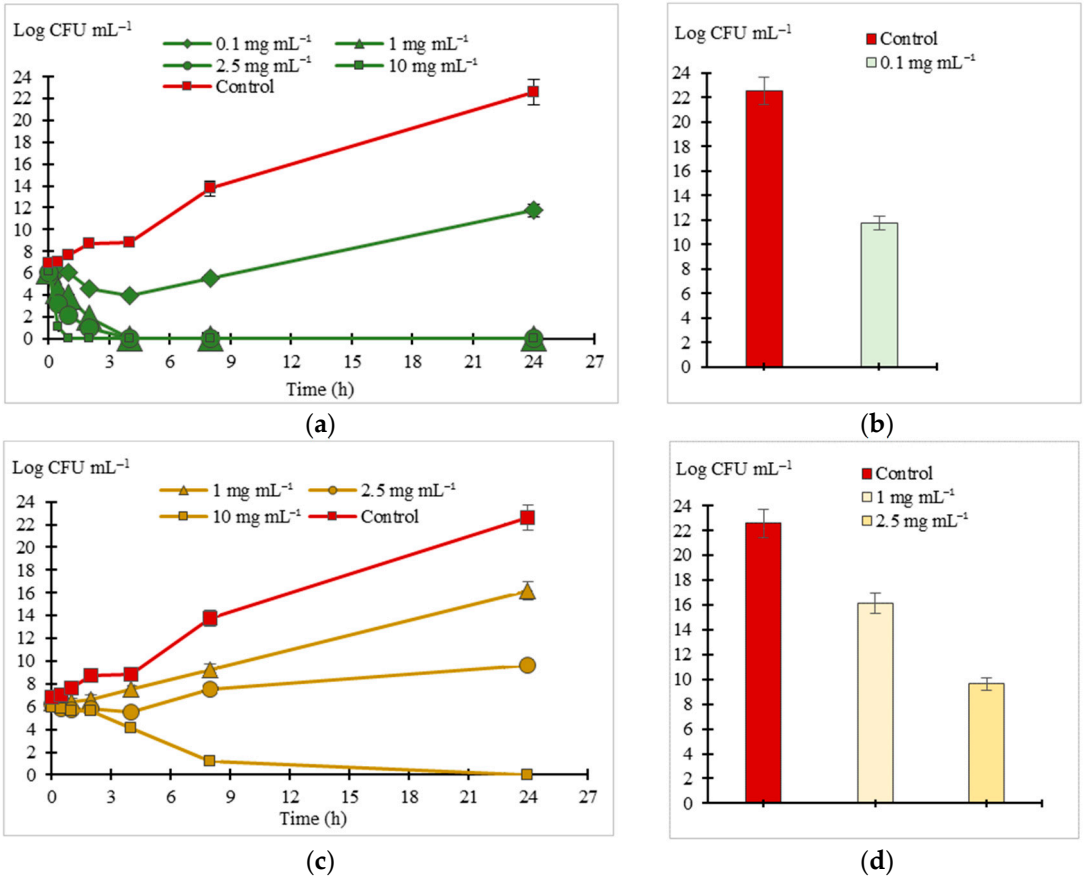

Figure 2. Time–kill curves for *S. aureus* ATCC 29213 at various concentrations of supercritical extracts obtained from: (**a**) *H. salicifolius* and (**c**) *H. tuberosus* with water as a co-solvent (CO_2 + H_2O extracts). Bacterial population density after 24 h exposure to various concentrations of the CO_2 + H_2O extracts obtained from: (**b**) *H. salicifolius* and (**d**) *H. tuberosus*. Mean values ± standard deviations are presented.

As presented in Figure 3, the CO_2 + H_2O extracts obtained from *H. salicifolius* and *H. tuberosus* differed in the total polyphenol content (TPC) expressed as gallic acid equivalents (GAE). This was 13.75 ± 0.50 mg GAE g^{-1} of *H. salicifolius* extract and 33.06 ± 0.80 mg GAE g^{-1} of *H. tuberosus* extract. Both extracts showed different antioxidant activity. *H. tuberosus* extract exhibited almost two-fold higher activity (EC_{50} = 0.332 ± 0.05 mg mL^{-1}) as compared to that of *H. salicifolius* (EC_{50} = 0.609 ± 0.29 mg mL^{-1}). However, a relationship was observed between the antioxidant potential of both extracts and TPC. It should be noted that despite two-fold lower TPC in the *H. salicifolius* extract than that in the *H. tuberosus* extract (Figure 3), activity of *H. salicifolius* extract against *S. aureus* ATCC 29213 was four-fold higher compared to *H. tuberosus* extract with MIC of 0.62 mg mL^{-1} or 2.5 mg mL^{-1}

(Table 3), respectively. In contrast, activity of both extracts against *E. coli* ATCC 25922 and *Candida* spp. strains was comparable (Table 3), irrespective of TPC (Figure 3).

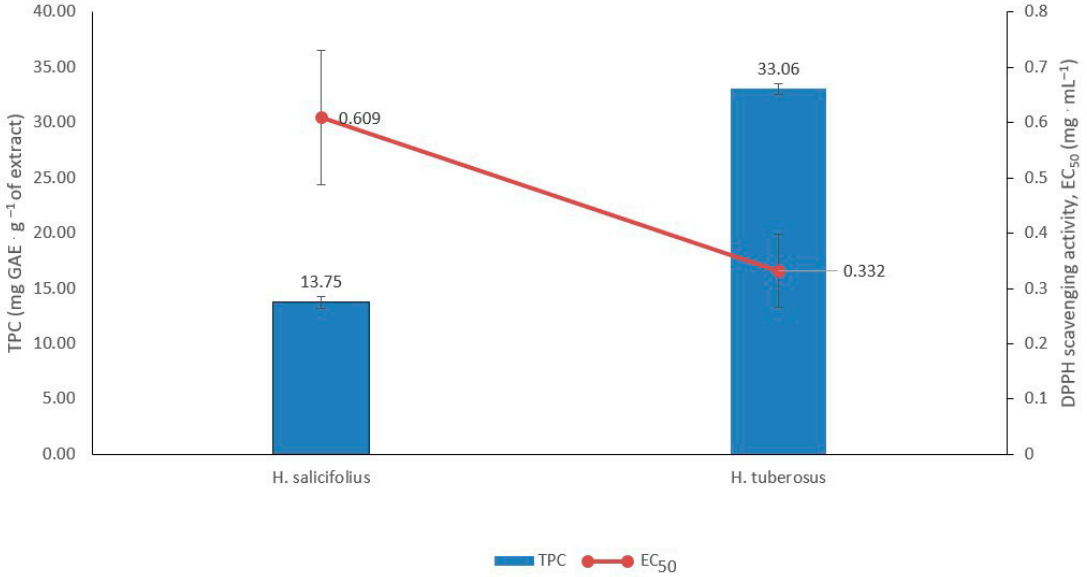

Figure 3. The total polyphenol content (TPC) in supercritical extracts obtained from *H. salicifolius* and *H. tuberosus* with water as a co-solvent (CO_2 + H_2O extracts) together with their antioxidant activity. Mean values ± standard deviation were presented.

The ATR–FTIR spectra of CO_2 + H_2O extracts from *H. salicifolius* and *H. tuberosus* are shown in Figure 4. Interpretation of these spectra was performed according to other authors [23–25]. The main vibrations of the characteristic groups were similar in both extracts; only slight diferrences were observed. At a wave-number of 3392 cm^{-1} for *H. salicifolius* extract and 3401 cm^{-1} for *H. tuberosus* extract, characteristic stretching vibrations were indicated that may have been induced from the OH group derived from carbohydrates proteins and polyphenols. In both analyzed spectra, cis C=C stretching was observed for both extracts at a wavenumbers of 3009 and 3008 cm^{-1}. The absorption bands around 2918 and 2849 cm^{-1} in the *H. salicifolius* extract spectrum and 2921 and 2851 cm^{-1} in the *H. tuberosus* extract spectrum may be due to asymmetric and symmetrical CH_2 stretching vibrations, respectively, while the band at a wavenumber of 1742 cm^{-1} is characteristic of the C=O stretching vibrations of aldehydes, ketones, and carboxylic acids. In the *H. salicifolius* extract, vibrations were recorded at a wavenumber of 1695 cm^{-1}; this band is characteristic of the vibrations of amide I (1600–1700 cm^{-1}), and the effect is related to the stretching vibrations of the C=O and C-N groups. Unconjugated stretching *cis* C=C at 1657 cm^{-1} was also observed. The band at 1462 cm^{-1} wavenumber was probably generated by a CH_2 scissor vibration. In contrast, the presence of the band at wavenumbers of 1369 cm^{-1} (*H. salicifolius* extract) and 1377 cm^{-1} (*H. tuberosus* extract) is characteristic of CH_3 symmetrical bending vibration. At wavenumbers 1239, 1167, 1096, and 1023 cm^{-1} and 1239, 1162, 1096, and 1030 cm^{-1}, respectively, for *H. salicifolius* and *H. tuberosus* extracts, stretching vibrations characteristic for the C-O groups were also seen. Furthermore, in the range of 970–969 cm^{-1} wavenumbers, characteristic vibrations for the groups *trans* double bonds (C=C) and *cis* double bonds (C=C) were revealed. In addition, the bands at 720 cm^{-1} are typical of the CH_2 groups. Moreover, at the wavenumber of 1323 cm^{-1} in the *H. salicifolius* extract, there was an absorption of the band that could be derived from amide III (C-N stretch) with a significant share of CH_2 carbohydrate residue. Table 4 shows major band assignments for the ATR–FTIR spectra of both CO_2 + H_2O extracts.

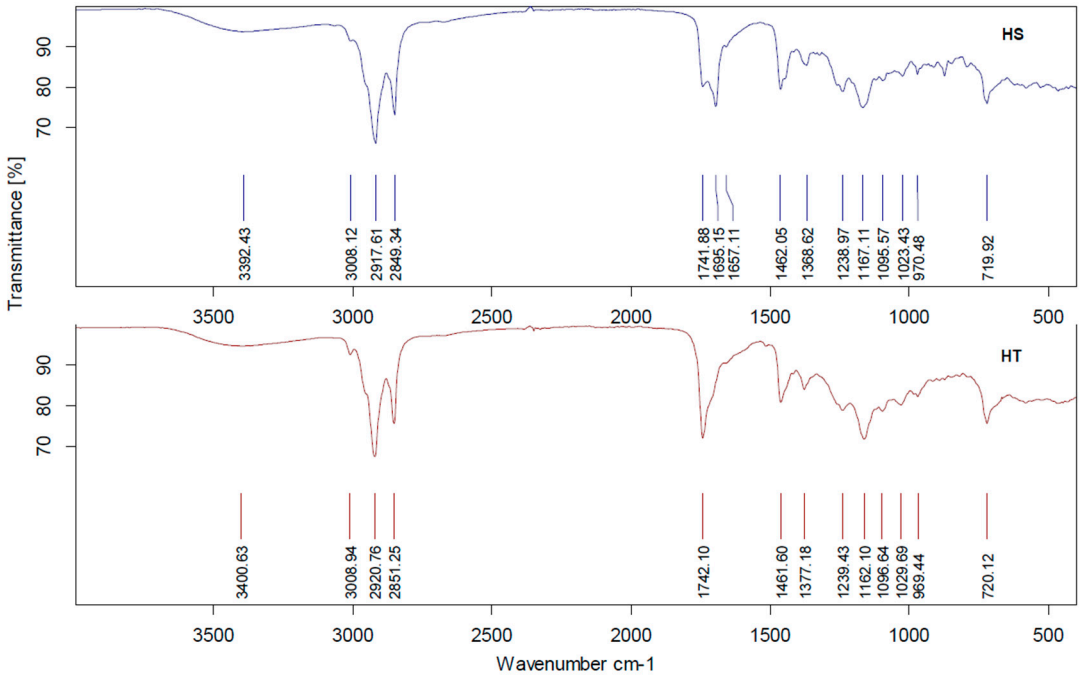

Figure 4. ATR–FTIR spectra of supercritical extracts obtained from *H. salicifolius* (HS) and *H. tuberosus* (HT) with water as a co-solvent (CO_2 + H_2O extracts).

Table 4. Major band assignments for the ATR–FTIR spectra of supercritical extracts obtained from *H. salicifolius* and *H. tuberosus* with water as a co-solvent (CO_2 + H_2O extracts).

Wavenumbers (cm^{-1})		Functional Group Vibration
H. salicifolius	***H. tuberosus***	
3392	3401	OH stretching (carbohydrates, proteins and polyphenols)
3008	3009	cis C=C stretching
2918, 2849	2921, 2851	asymmetric and symmetric stretching vibration of CH_2 group
1742	1742	C=O stretching (aldehydes, ketones, and carboxylic acids)
1695	-	C=O and C-N stretching vibrations
1657	-	unconjugated cis C=C
1462	1462	CH_2 scissor vibration
1369	1377	CH_3 symmetrical bending vibration
1239, 1167, 1096, 1023	1239, 1162, 1096, 1030	C-O stretching vibration
970	969	trans double bonds (C=C) and cis double bonds (C=C)
720	720	bending (rocking) of -$(CH_2)_n$-, -HC-CH-(cis)

4. Discussion

Data presented in this paper showed that CO_2 + H_2O extracts obtained from both *Helianthus* species possessed antimicrobial potential, including activity against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*), as well as yeasts (*C. albicans* and *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 [13–15]. 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 [26,27]. According to the literature data [9–11], extracts from

H. tuberosus leaves might be a promising source of natural fungicides active against several phytopathogens, among them caffeoylquinic acid derivatives. However, 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. Of note, there is no literature data on bioactivity and chemical composition of the supercritical extracts obtained from the aerial parts of *H. tuberosus* and *H. salicifolius* using water as co-solvent (CO_2 + H_2O extracts). This is the first report.

The antistaphylococcal activity of CO_2 + H_2O extracts obtained from *H. salicifolius* and from *H. tuberosus* should be underlined. *S. aureus* is known to be an important pathogen related to skin and soft tissue, as well as to food-borne infections [28]. In this paper, we found higher antistaphylococcal effect of *H. salicifolius* CO_2 + H_2O extract, as compared to that of *H. tuberosus* CO_2 + H_2O extract. In contrast, it was found that TPC was higher in *H. tuberosus* extract, in comparison to that in *H. salicifolius* extract. Yuan et al. [29] showed that TPC in aqua ethanolic extracts from *H. tuberosus* leaves was 101.07 mg GAE g^{-1} of dry extract. More detailed studies by Showkat et al. [30] revealed that TPC in *H. tuberosus* was dependent on the plant organ. They determined higher TPC, expressed as mg GAE g^{-1} of dry substance, in ethanolic extracts from leaves (7.9–11.1) than in flower (4.0–5.3), tuber (2.8–3.8), and stem (0.9–1.7) extracts. However, these authors found the overestimation of TPC in the extracts from various organs ranging from 65% (in flowers) to 94% (in stems) and used the Folin–Ciocalteu assay, applying the correction for ascorbic acid.

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 [31]. Data presented in this paper suggest that the antistaphylococcal activity of both CO_2 + H_2O extracts, especially that from *H. salicifolius*, may be due to the content of other plant secondary metabolites such as sesquiterpene lactones [32]. These compounds can be regarded as one of the most prevalent and biologically significant classes of plant secondary metabolites, including that in plants from Asteraceae family, e.g. *H. tuberosus* [29]. Sesquiterpene lactones have been assumed to be potent antimicrobials. Some are also considered to be antioxidants [33,34].

The antimicrobial properties of plant-derived products are generally accompanied by a confirmed antioxidant capacity [29,31–34]. In this paper, we found higher antioxidant effect of *H. tuberosus* CO_2 + H_2O extract together with its higher TPC, as compared to those of *H. salicifolius* CO_2 + H_2O extract. These data suggest that the antioxidant properties of CO_2 + H_2O extracts studied may be related to polyphenols content, which is in agreement with the literature data [35,36]. The DPPH assay included in this study for the determination of antioxidant activities of the CO_2 + H_2O extracts from both *Helianthus* species was also used by other authors [30,37,38] in studying the radical scavenging activity of *H. tuberosus*. Showkat et al. [30], for example, showed significant correlation between TPC and radical scavenging activity of ethanolic extracts from *H. tuberosus* leaves. These authors revealed, similarly to Yuan et al. [37] and Nizioł et al. [38], that aqua-ethanolic or ethanolic extracts from *H. tuberosus* leaves, in comparison to those from other organs of this plant (e.g. tubers), possessed higher radical scavenging activity (with EC_{50} about 0.075–0.25 mg mL^{-1}) and could be a potential source of natural antioxidants.

The ATR–FTIR spectroscopic imaging is a suitable technique, not only as a method of identity confirmation, but also for detecting and identifying molecular components in a complex plant matrix [23]. Preliminary phytochemical analysis of both CO_2 + H_2O extracts by ATR–FTIR indicated the presence of similar main vibrations of the functional groups typical for phytoconsituents possessing bioactivity such as polyphenols, aldehydes, ketones, or carboxylic acids [23–25].

5. Conclusions

The presented data suggest that supercritical extracts with water as a co-solvent obtained from the aerial parts of *H. salicifolius* and *H. tuberosus* collected in summer period appeared to be a promising source of natural compounds with biocidal effect. They

possessed antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) species (MIC = 0.62–5 mg mL^{-1}), as well as antifungal activity against yeasts from *Candida* genus (MIC = 5–10 mg mL^{-1}). It is worth notifying their antistaphylococcal activity (MIC = 0.62–2.5 mg mL^{-1}). These extracts may be also regarded as natural potential antioxidants (EC_{50} = 0.332–0.609 mg mL^{-1}). The ATR–FTIR spectra of both extracts showed similar main vibrations of the functional groups typical for phytoconstituents possessing bioactivity. The obtained data, together with those from literature, suggest that these extracts and their isolated bioactive compounds may be used as conservants in cosmetics and/or natural preservatives in food. However, further studies are needed to confirm the obtained results, to define and to quantify constituents present in both extracts, as well as to identify specific applications of supercritical extracts and their phytoconstituents from biomass of these two plant species.

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