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Supercritical CO_2 extraction of natural antibacterials from low value weeds and agro-waste

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ABSTRACT

The suitability of supercritical carbon dioxide ($scCO_2$) to extract cosmetic-friendly preservatives from low value weeds and agro-waste was investigated. The essential oils extracted from *Humulus Lupulus* and *Datura Stramonium* (leaves and flowers) were analysed in search for potential antibacterial compounds. Their antibacterial activity was evaluated against *Escherichia coli* in view of using the extracts as preservatives in cosmetic formulations.

The composition and antibacterial activity of the $scCO_2$ extracts were compared to the ones obtained by Soxhlet extraction with ethanol. The two methods resulted very different in terms of yields, selectivity, composition, and antibacterial properties of the extracts.

In general, $scCO_2$ was better for the recovery of preservative compounds since it led to the selective extraction of volatile oils composed mainly by terpenes, terpenoids, fatty acids, and bitter acids.

1. Introduction

Most personal-care formulations are organic- and water-based and, thus, easily degradable by microorganisms. To prevent microbial proliferation, cosmetics contain preservatives. The search for improved biocompatibility and the emergence of stringent regulations on cosmetic formulations [1] are prompting replacement of the commonly used synthetic preservatives (parabens, phenolics, formaldehyde releasers, isothiazolinones, phenoxyalcohols, quaternary ammonium compounds and organic acids) with natural antiseptic and preservative compounds. Plants and, more specifically, their essential oils are considered as one of the main sources of natural preservatives. These substances are volatile liquids with varying viscosity, typically constituted by monoterpenes, terpenes, terpenoids and low molecular weight aromatic or aliphatic compounds [2–5].

Given the wide-ranging interests of our group in developing biomass-derived platform chemicals [6–13], we here investigate the supercritical CO_2 ($scCO_2$) extraction of natural preservatives from *Humulus lupulus* (hops) and *Datura stramonium* (jimsonweed): these plants were chosen for their widespread availability and low value, belonging to the class of agricultural and agroindustrial wastes. Both contain molecules with proven antiseptic properties [14–25], amongst which we focused on compounds with potential preservative functions suitable for cosmetic formulations. Examples of such compounds include fatty acids, terpenes and terpenoids [4,16,17,26,27].

Humulus lupulus is a perennial herbaceous species of flowering plants belonging to the *Cannabaceae* family. Over the last decades, essential oils from *Humus lupulus* flowers or leaves were isolated by conventional or microwave-assisted hydrodistillation [28–30], $scCO_2$ extraction [30–35], solid phase microextraction [36] or by Soxhlet extraction with organic solvents [35]. Hops flowers have been known since ancient times not only to preserve and give flavour to beer, but also as sedatives, against sexual disorders, as appetisers and topically against neuralgic, rheumatic or arthritic pain. To date, due to the crucial importance in beer production, the preservative properties of the hops flowers extracts have been extensively studied and the bitter acids seem to be the main active compounds against Gram-positive bacteria [14,16,25]. On the contrary, the hops leaves are usually discarded as a waste. Since however it is known that the leaves contain volatile compounds as well, their valorisation seems to be an interesting topic to explore in the context of the circular economy and of a more efficient production scheme [28,29,31,37].

Datura stramonium (jimsonweed) is an annual, invasive, night-blooming plant of the *Solanaceae* family which contains biologically-

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active molecules in all its parts. These compounds include saponins, sterols, flavonoids, coumarins, quinines and a variety of alkaloids – molecules with a potent physiological activity – some of which can lead to poisoning and death even in small doses [38,39]. This invasive species is, therefore, often an undesired presence, especially in cultivated crops, where it is discarded as agricultural waste. Many studies have been conducted on its toxicity and pharmacology [40–42], but there are only reports focused on the antibacterial activity of jimsonweed extracts obtained through maceration with organic solvents and/or water [18,20,22,23,43].

The present work aims at valorising these two common agricultural wastes, *Humulus lupulus* leaves and *Datura stramonium* as a whole, by upgrading them to sources of antibacterial molecules *via* a green $scCO_2$ extraction protocol. To this end, the supercritical extraction methodology was first optimized with the hops cones and then implemented to extract the volatile fractions of the hops leaves and of jimsonweed. The chemical composition and antibacterial activity of the extracts were then investigated to identify the bioactive compounds. The composition and the preservative efficacy of the extracts were further compared with those obtained by conventional Soxhlet extraction with ethanol as the solvent. The latter being known as a poorly selective albeit intensive extraction method, hardly sustainable on the industrial scale [44,45].

2. Materials and methods

2.1. Apparatus and chemicals

All chemicals and solvents were purchased from commercial sources and used without further purification. Ethanol (pharma-grade, purity ≥99.8%), *n*-dodecane (purity ≥99.0%) and the deuterated solvents (isotopic purity atom% D: acetone-d_6 ≥99.9, methanol-d_4 ≥99.8, chloroform-d ≥99.8) were all provided by Sigma-Aldrich. Supercritical CO_2 extractions were performed in a laboratory apparatus consisting of a Jasco PU-2080 − CO2 Plus delivery Pump and a Jasco 2080 Plus Automatic Back Pressure Regulator used for the pressure control. The connecting stainless tubes and the different reactors were heated by a Chrompack CP-9003 oven. The GC-FID was a HP 5890 equipped with an Elite-624 capillary column (30 m, internal diameter 0.32 mm, film 0.18 μm,) coupled with a FID detector. The GC–MS (EI, 70 eV) was a 6890 N Network GC System coupled with a 5973 inert Mass Selective Detector (both from Agilent) equipped with a HP5 column (30 m, internal diameter 0.25 mm, film 0.25 μm, 5% diphenyl 95% dimethyl siloxane). The following conditions were used. Carrier gas: He (1.2 mL/min, constant flow); inlet temperature 250 °C; split mode; ratio 30:1; detector temperature: 280 °C; ramp rate: 10 °C min^{-1}; final T: 230 °C (20 min). ^1H and $^{13}C\{^1H\}$ NMR spectra were collected at 25 °C on a Bruker Ascend 400 operating at 400 MHz for ^1H and 100 MHz for ^{13}C, or on a Bruker UltraShield 300 operating at 300 MHz for ^1H and 75 MHz for ^{13}C. For ^1H and $^{13}C\{^1H\}$ NMR the chemical shifts (δ) have been reported in parts per million (ppm) relative to the residual non-deuterated solvent as an internal reference and are given in δ values downfield from TMS.

2.2. Sample preparation

The aerial parts of *Datura stramonium* and *Humulus lupulus* were hand-harvested from pesticide-free organically maintained fields (Veneto, Italy) and manually chopped into 1 − 2 cm pieces. The samples were then dried at room temperature for 2 days under air, milled with a spice grinder to 0.5 − 1 mm pieces and then stored at 4 °C (Figs. 1 and 2).

2.3. Soxhlet extraction procedures

The Soxhlet apparatus (containing a cellulose thimble) was filled with 3 g of dried and ground biomass and 150 mL of ethanol; the solvent was refluxed for 24 h. For the qualitative and quantitative characterisation of the volatile fraction, the solution was analysed by GC-FID/GC–MS, using *n*-dodecane (2 mg/mL) as internal standard. The solvent was then evaporated under reduced pressure to determine the extraction yield ($Y_{extract} = wt_{extract}/wt_{dry\ biomass}$ %).

2.4. Supercritical fluid extraction procedures

The $scCO_2$ extraction experiments were conducted on an analytical scale supercritical extraction unit (internal volume of approximately 10 cm^3, bed diameter 3/8″ and bed height of 15 cm) using $scCO_2$ as solvent. The chamber was loaded with approximately 3 g of dry, ground biomass and extracted with a constant flow rate of $scCO_2$ (5.0 $cm^3\ min^{-1}$) at 300 bar and 70 °C for 5 h. The extracts were collected by venting in ethanol at ambient temperature and pressure. Following removal of ethanol at low temperature under a gentle flow of nitrogen, the extracts were gravimetrically quantified ($Y_{extract} = wt_{extract}/wt_{dry\ biomass}$ %) at the end of each run. For the qualitative and quantitative analysis of the volatile fraction, an ethanol solution of a known quantity of extract was analysed by GC-FID/GC–MS, using *n*-dodecane (0.5 mg/mL) as internal standard.

2.5. Chromatographic analysis of the extracts

The extracts were analysed by GC–MS. The identification of the key components was performed by using the NIST database [46], by comparison with the existing literature [20,24,28–33,36,38,39] and, whenever informative, by integrating this MS information with the NMR spectra (See supporting info). For some compounds whose NIST reliability range did not allow to assign a structure with certainty, the proposed structures are tentative.

Quantification was performed by GC–FID under the same analytical conditions used for the GC-MS system and with the same chromatographic column. For each chromatogram the peak area (A_x) was used to estimate the quantity of each compound (Q_x)with reference to the internal standard peak area (A_{std}) and its quantity added to the extract (Q_{std}), using the following formula:

$$Q_x = \frac{A_x}{A_{std}} Q_{std}$$

The estimated total amount of volatile compounds – defined as all the species with a GC retention time < 20 min at GC-FID (Σ Qx) – was used to calculate the "yield of volatile compounds" ($Y_{volatile}$) referring to the total weight of the recovered extract (Q_{ext}), with the following formula:

$$Y_{volatile} = \frac{\sum_{x=1}^{n} Q_x}{Q_{ext}} * 100$$

2.6. Evaluation of the antibacterial activity

The antibacterial properties of the *Humulus Lupulus* and *Datura Stramonium* extracts were tested against *Escherichia coli*, as a standard model. The bacteria were grown overnight using LB medium on a 15 mL falcon shaken at 250 RPM at 37 °C. The bacteria were then diluted 1/10, transferred on a 96-well plate and finally treated with five different concentrations of the extracts in the range between 40 and 2.5 mg/mL using two-fold dilutions. The culture was grown for 6 h, then the antibacterial activity was evaluated by CellTiter-Glo® Luminescence Assay (Promega, Madison, WI, USA) with Synergy H1 instrument (Biotek, Milan, Italy).

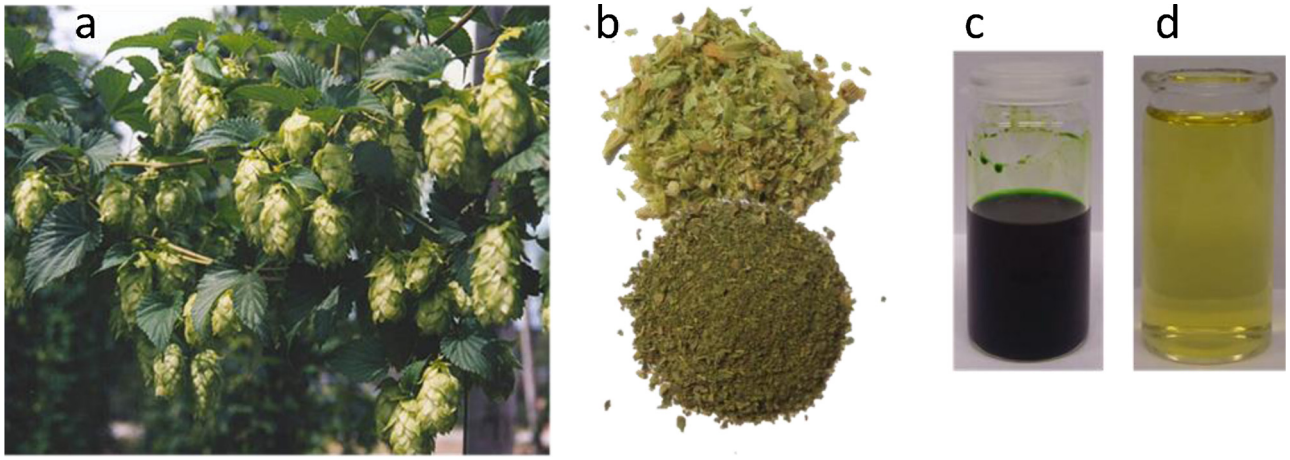

Fig. 1. *Humulus Lupulus* (a) (hops): dried and ground flowers and leaves (b), ethanolic extract (c) and $scCO_2$ extract (d) of the leaves.

3. Results and discussion

3.1. Humulus lupulus

To identify the optimal $scCO_2$ extraction conditions, preliminary experiments were conducted using hops flowers (3 g for each test), the results are shown in Table S1 and indicate that the extraction yields increased with increasing pressure, temperature, time and volumetric CO_2 flow. The highest final yield (8.6%) was achieved at 70 °C, 300 bar, 5 mL/min of $scCO_2$ flow and after 5 h of extraction (entry 5, Table S1). Interestingly, the curves obtained for the extraction with $scCO_2$ at different pressures and flows showed a behaviour which correlates with the density of the fluid and the space velocity, used as reactor design parameter (see Fig. 3 a,b and Table S1).

Under the optimized conditions, the $scCO_2$ extraction procedure was extended to the hops leaves, usually discarded as a waste after the harvest. The yields of the extracts with $scCO_2$ were further compared to the ones obtained by Soxhlet extraction using ethanol as the solvent for 24 h. Ethanol, not hexane, was selected as the extraction solvent for the comparison. The main reason being that the extracts are here proposed as antimicrobial additives for cosmetics. While ethanol is allowed, hexane is banned due to its toxicological properties. In addition, we have used hexane for other biomass extractions and the yields and selectivity are very similar to those obtained with $scCO_2$. The 24 h extraction ensures complete extraction of all the components. The results are reported in Table 1.

As expected based on the solvent properties, the $scCO_2$ extraction of the flowers (Table 1, entry 1) yielded a pale yellow, clear extract with a lower extraction yield by weight (9% $_{wt/wt\ biomass}$) compared to ethanol (Table 1, entry 2; 27% $_{wt/wt\ biomass}$) but with a higher selectivity towards the volatile fraction: 26% *versus* 4% $_{wt/wt\ extract}$. The ethanolic high-yield and low-selectivity were reflected in the dark-brown colour and turbidity of the solution. Similar behaviour was observed with the hops leaves: using $scCO_2$, 4% $_{wt/wt\ biomass}$ yield of a pale yellow-green coloured oil with a high selectivity of volatiles (18% $_{wt/wt\ extract}$) was obtained; while a higher yield of brown coloured oil (14% $_{wt/wt\ extract}$) but with a low content of volatiles (2% $_{wt/wt\ extract}$), was obtained using ethanol (Table 1, entry 3 and 4). The differences in yield and selectivity between $scCO_2$ and ethanol are not surprising. However, considering our objective of obtaining antimicrobial compounds, the higher selectivity with $scCO_2$ is preferable over the higher yield of a more heterogenous mixture of substances obtained with ethanol.

The solutions were then analysed by GC–MS to identify the main components of the volatile fractions by matching their spectra with those of the Mass Spectra Library (NIST 2.0) and with literature data [24,28–33,36]. The structural analysis highlighted the presence of molecules (Figure S1) of the terpene (**1-7**), terpenoid (**8–10**), fatty acid (**11–13**) and bitter acid (**14–18**) classes, whose distribution was

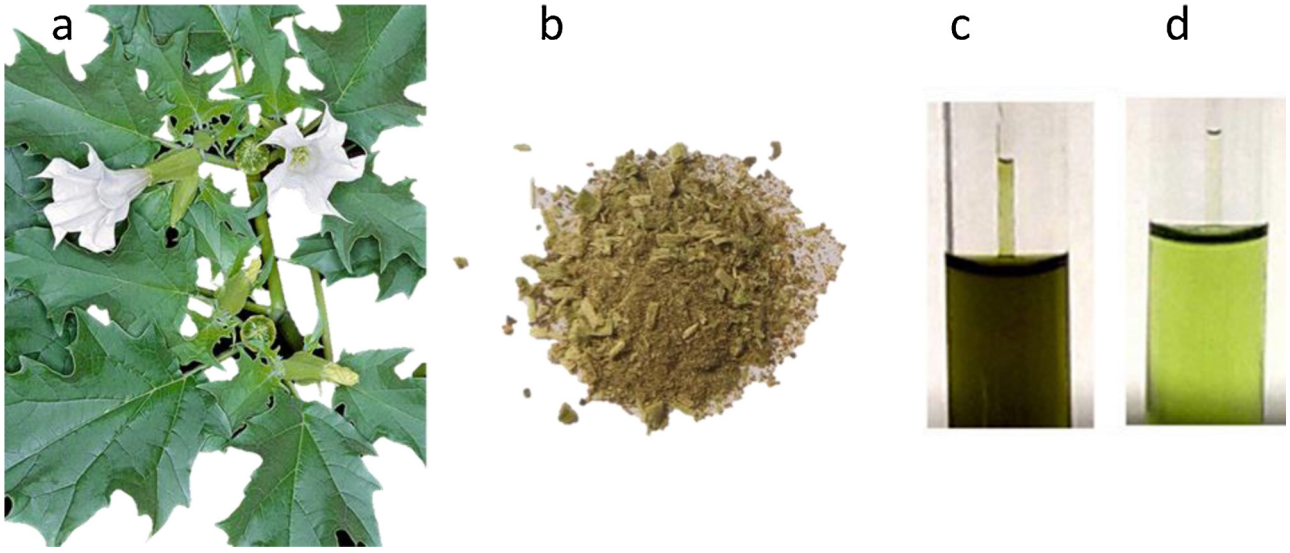

Fig. 2. *Datura Stramonium* plant (a), dried and ground biomass (b), ethanolic extract (c) and $scCO_2$ extract (d) of jimsonweed.

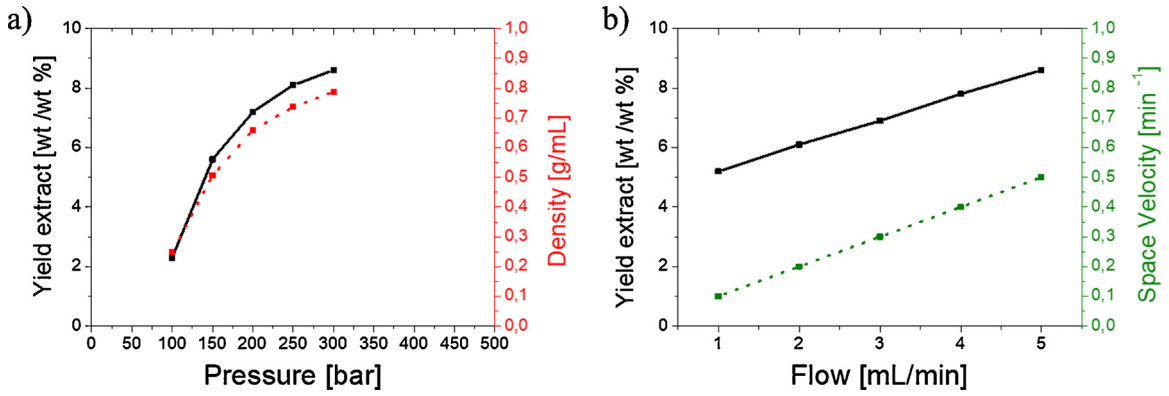

Fig. 3. Effect of a) the pressure and b) the volumetric flow on the extraction yields of the *Humulus lupulus* flowers and their correlation with the CO_2 density and the space velocity.

Table 1
Extraction yields of hops flowers and leaves with $scCO_2$ and ethanol as solvents.

Entry	Hops	Solvent	$Y_{extract}$ (%) [c]	$Y_{volatile}$ (%) [d]
1	Flowers	$scCO_2$ [a]	8.6	26
2	Flowers	Ethanol [b]	27	4
3	Leaves	$scCO_2$ [a]	4	18
4	Leaves	Ethanol [b]	14	2

Extraction conditions: a) 3 g of dry and ground *Humulus lupulus* flowers or leaves, $scCO_2$ at 70 °C, 300 bar, 5 mL/min, 5 h. b) 3 g of dry and ground *Humulus lupulus* flowers or leaves in Soxhlet, 150 mL of ethanol, reflux, 24 h. c) Amount of compounds extracted by weight of sample (% $_{wt\ /wt\ biomass}$). d) Percentage of volatile compounds in the extract as determined by GC-FID using *n*-dodecane as internal standard (% $_{wt/wt\ extract}$).

dependent on the extraction method. The hops bitter acids in the flowers consisted of two related series of 6C-ring and 5C-ring compounds having isobutyroyl acyl (**14–15**) or isovaleroyl (**16–18**) side chains.

The percentages of the predominant volatile components present in all the extracts of the hops flowers and leaves, are given in Table 2. Other components not detected by GC-FID are presumably heavier lipidic fractions. The volatile oils obtained by $scCO_2$ extraction of the flowers comprised mainly β-myrcene (**1**), β-farnesene (**2**), β-pinene (**3**), β-caryophyllene (**4**), cadinene conformers (**5-7**) and bitter acids (**15-18**). In particular, the compounds extracted in higher percentage were two bitter acids: hulupone (**17**) in 8.2% and lupulone in 8.7% (**18**), both already known for their antiseptic activity [14,16,25].

Table 2
Identification of the main volatile components of the essential oils from hops flowers and leaves.

Compound	Flowers		Leaves		
	$scCO_2$	Soxhlet	$scCO_2$	Soxhlet	Retention time (min)
	$\%_{w/w\ extract}$				
β-myrcene **(1)**	0.3	0.2	–	–	6.47
β-farnesene **(2)**	1.0	0.2	–	0.02	10.40
β-pinene **(3)**	0.5	–	–	–	6.46
β-caryophyllene **(4)**	0.5	0.1	0.2	0.01	10.26
γ-cadinene **(5)**	0.1	–	–	–	10.60
β-cadinene **(6)**	1.0	0.3	–	–	10.70
α-cadinene **(7)**	1.3	0.4	–	–	10.75
phytol **(8)**	–	–	0.2	0.92	14.15
α-humulene **(9)**	0.3	–	–	–	11.72
cadinol **(10)**	0.1	–	–	–	11.72
azelaic acid **(11)**	–	–	0.5	–	11.43
oleic acid **(12)**	–	–	0.1	–	13.10
linoleic acid **(13)**	–	–	0.6	0.02	14.31
cohumulinic acid **(14)**	–	–	2.3	–	12.07
dehydrocohumulinic acid **(15)**	1.3	–	2.2	–	13.20
humulinic acid **(16)**	1.3	–	2.9	–	13.75
hulupone **(17)**	8.2	–	–	–	17.17
lupulone **(18)**	8.7	1.1	–	–	18.43

On the other hand, the $scCO_2$ extracts of the hops leaves contained β-Caryophyllene (**4**), phytol (**8**), fatty acids (**11-13**) and 5C-ring bitter compounds (**14-16**). In the oil obtained from the leaves, the main components were bitter acids, in particular cohumulinic (2.3%, **14**), dehydrocohumulinic (2.2%, **15**) and humulinic acid (2.9%, **16**), that have a certain antibacterial activity [14,16,25].

In comparison, both for flowers and leaves, the ethanol extracts resulted lower in terms of both amount and variety of volatile compounds.

It is worth mentioning that all these classes of compounds have been reported as antiseptics [5,14,16,17,24,26,27,47–49]. Therefore, since most of them were found preferentially in the $scCO_2$ extracts, this was considered as the most suitable extractive technique to recover preservative compounds from hops. Moreover, it is interesting to note that with $scCO_2$ also the waste leaves represent a sustainable source of antibacterial compounds. The $scCO_2$ extracts of the hops flower were richer in terpenes (**1–7**) and bitter acids (**15–18**) while the hops leaves extracts were composed mainly by fatty acids (**11–13**) and bitter acids (**14-16**). These differences affect the antibacterial activity of the extracts as discussed in Section 3.3.

3.2. *Datura stramonium (jimsonweed)*

Extractions with $scCO_2$ and ethanol were then carried out on dried and milled flowers and leaves of *Datura stramonium*, and the yields are summarised in Table 3. The $scCO_2$ extraction of the flowers lead to a light green, clear extract with a lower extraction yield (1.2% $_{wt/wt\ biomass}$)

Table 3
Extraction of *Datura stramonium* flowers and leaves with $scCO_2$ as solvent and with ethanol using the Soxhlet apparatus.

Entry	*Datura stramonium*	Method	$Y_{extract}$ [c]	$Y_{volatile}$ [d]
1	flowers	$scCO_2$ [a]	1.2	24.8
2	flowers	Soxhlet [b]	22.0	1.5
3	leaves	$scCO_2$ [a]	2.3	16.4
4	leaves	Soxhlet [b]	21.9	3.3

Extraction conditions: a) 3 g of dry and ground *Datura Stramonium* flowers or leaves, $scCO_2$ at 70 °C, 300 bar, 5 mL/min, 5 h. b) 3 g of dry and ground *Datura Stramonium* flowers or leaves in Soxhlet, 150 mL of ethanol, reflux, 24 h. c) Amount of compounds extracted by weight of sample (% $_{wt\ /wt\ biomass}$). d) Percentage of volatile compounds in the extract as determined by GC-FID using *n*-dodecane as internal standard (% $_{wt/wt\ extract}$).

Table 4
Identification of the main FAs components of the essential oils from *Datura stramonium* flowers and leaves.

Compound	Flowers		Leaves		
	$scCO_2$	Soxhlet	$scCO_2$	Soxhlet	Retention time (min)
	% $_{w/w\ extract}$				
phytol **(8)**	2.5	–	5.3	0.4	14.15
azelaic acid **(11)**	0.1	0.03	0.3	0.1	11.43
oleic acid **(12)**	–	–	–	0.1	13.10
linoleic acid **(13)**	3.1	–	–	–	14.31
1-tridecene **(19)**	1.3	–	0.8	0.2	9.95
16-heptadecenal **(20)**	0.1	–	–	–	12.59
γ - linolenic acid **(21)**	5.1	0.09	2.3	0.3	14.32
stearic acid **(22)**	2.9	0.05	–	–	14.45
diethyl adipate **(23)**	–	0.03	–	–	9.91
capryl alcohol **(24)**	0.1	–	–	–	11.94
palmitic acid **(25)**	5.5	0.15	3.1	0.4	13.19
N-acetyltyramine **(26)**	0.2	–	–	–	13.60
conhydrine **(27)**	–	0.05	–	–	8.91
atropine **(28)**	–	–	0.4	0.3	15.29
scopolamine **(29)**	0.4	–	0.2	–	16.74

compared to ethanol (22%$_{wt/wt\ biomass}$) but with high selectivity towards the volatile fraction: 24.8% compared to 1.5% $_{wt/wt\ extract}$, respectively (Table 3, entries 1 and 2). The same trend was once more observed for the leaves, in this case however, the $scCO_2$ extract was characterized by a slightly lower selectivity towards the volatile fractions (from 24.8–16.4% $_{wt/wt\ extract}$ for flowers and leaves respectively).

As can be seen in Table 4, both $scCO_2$ and ethanol extracted preferentially phytol (**8**) and fatty acids (**11–13**, **21 − 25**). Except for the poisonous alkaloids (N-acetyltyramine (**26**), conhydrine (**27**), atropine (**28**) and scopolamine (**29**), [20,21,38,39,41,42] that were however extracted with very low selectivity, all the detected compounds reasonably possess the desired preservative properties [18,20–23,41,43], making them suitable for cosmetic formulations. Looking at the $scCO_2$ extracts it is interesting to note that they have a high percentage of fatty acids (linoleic acid **13**, γ-linoleic acid **21**, stearic acids **22** and palmitic acid **25**), compounds known to have antibacterial activity [26,27,48,52].

3.3. Antibacterial activity

Antibacterial action depends strictly on the chemical composition of

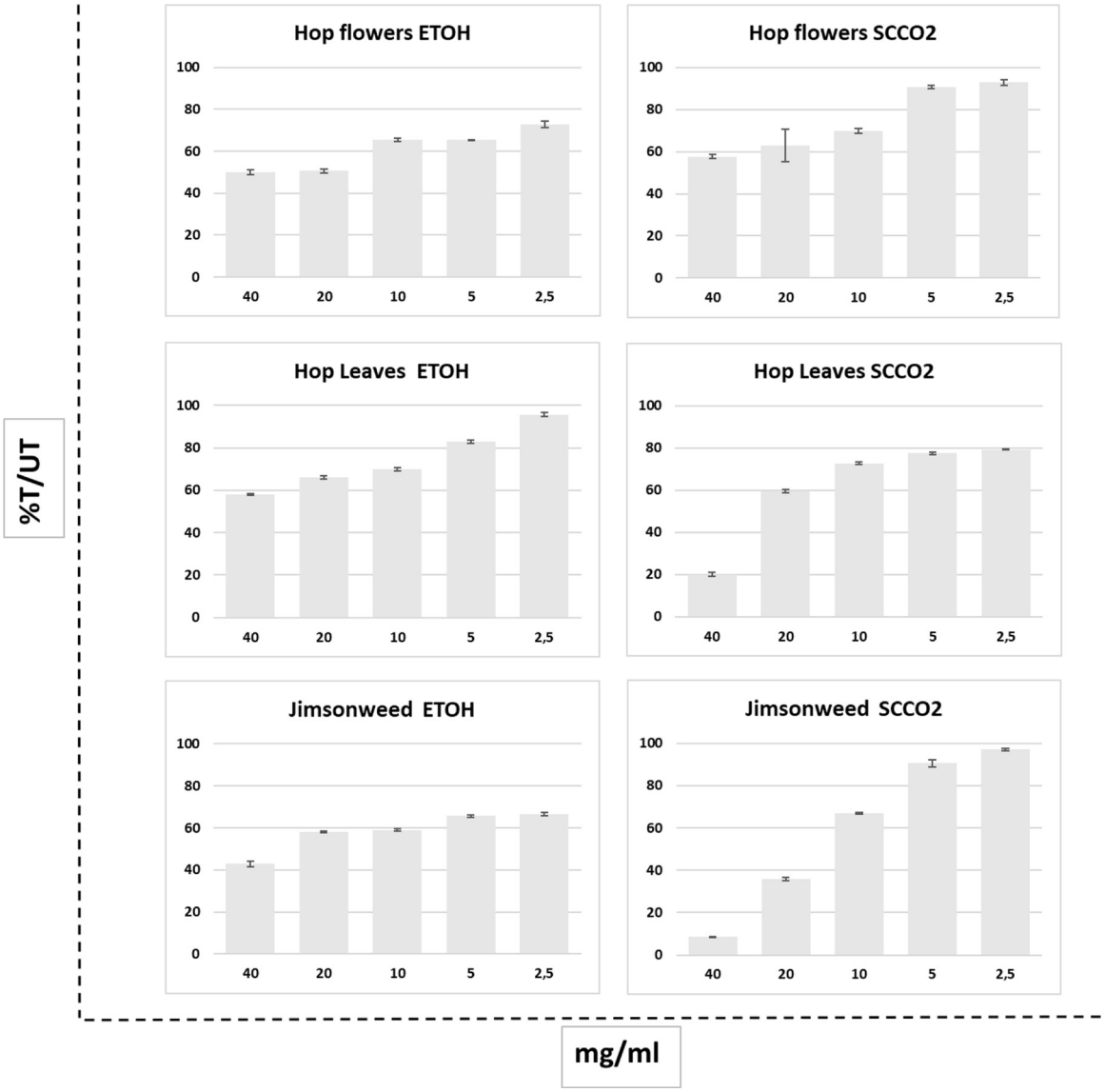

Fig. 4. Antimicrobial activities on *Escherichia Coli* of hop and jimsonweed extracts using from ethanol and $scCO_2$ as solvents. On Y axis, the percentage of cell viability is reported of treated (T)/untreated (UT) samples. The values on X axis are in mg/mL. Columns represent average values and error bars are standard deviations.

the extracts and is not due to a unique mechanism but, instead, depends on a cascade of reactions. In general, essential oils inhibit the growth of bacterial cells and the production of toxic bacterial metabolites, affecting both the external membrane of the cell and the cytoplasm. Bacterial disruption is due to the hydrophobicity of the components of the natural oils which can penetrate the microbial cell and cause alterations in its structure and functionality [4,47,50,51].

Amongst the most effective biocides contained in essential oils are fatty acids, bitter acids, terpenes and terpenoids. The antibacterial action of fatty acids targets the cell membrane by modifying the permeability of the cell wall and disrupting the electron transport chain and the oxidative phosphorylation. In addition, fatty acids may also inhibit enzyme activity, impair nutrient uptake, generate peroxidation and auto-oxidation degradation products or direct lysis of bacterial cells [52]. In this context, it has been demonstrated that unsaturated fatty acids are more active against bacteria than saturated ones [27,48]. The action of bitter acids towards Gram-positive bacteria is similar to that of fatty acids: they are able to leak the primary membrane due to the interaction of the hydrophobic parts of the molecules with the phospholipid bilayer of the cell wall [16]. Toxic effects on membrane structure and function have been invoked also to explain the antimicrobial action of terpenes and terpenoids. Their lipophilic character, in fact, promotes their partitioning into the membrane structure resulting in an expansion of the cell wall and in an increase of the membrane fluidity and permeability. These effects inhibit the respiration and alter the ion transport processes due to the structural modification of the membrane embedded proteins [17,51,53].

Taking into consideration the above explained peculiar features of the classes of compounds present on the recovered oils, the antibacterial properties of the *Humulus Lupulus* and *Datura Stramonium* extracts were tested against *Escherichia coli,* a standard model for bacteria studies. The extracts were tested on bacterial cells starting from a concentration of 40 mg/mL with a serial two-fold dilution. The results reported in Fig. 4 represent the percentage of cell viability at different extract concentrations (mg/mL). Overall, ethanol derived extracts are less active than $scCO_2$ derived extracts. In detail, jimsonweed $scCO_2$ extracts inhibit the growth of bacterial cells more than 80% and hop leaves extracts more than 75%. The equivalent ethanol derived extracts never exceed 60% of growth inhibition. Extracts from hop flowers are weakly active and their activity is independent from the extraction methods.

4. Conclusions

The results obtained in this study demonstrate that $scCO_2$ extraction of low-value agro-waste such as hops leaves, and jimsonweed represent a viable source of natural preservatives and may contribute an additional step in the circular economy context. Although the $scCO_2$ extraction gave lower extraction yields ($8.6-4\%_{\text{ w/w biomass}}$ for *Humulus Lupulus* and 1.2–2.3% $_{\text{w/w biomass}}$ for *Datura Stramonium* flowers or leaves respectively) compared to ethanol extraction with the Soxhlet, $scCO_2$ always reached the highest selectivity (up to 26% $_{\text{w/w extract}}$) towards the light fraction containing potentially antibacterial molecules. Additionally, the potential loss of part of the volatile components extracted with $scCO_2$ during workup and the lower yield was considered acceptable in the present context, considering that our objective was to obtain antimicrobial compounds by a viable procedure. The extracts were tested towards *E Coli*, showing that the $scCO_2$ extracts are more active as antibacterial compared to the ethanol ones.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcou.2020.101198.

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