PHYTOCHEMICAL STUDY AND ANTIMICROBIAL ACTIVITY OF *Cichorium intybus* L. AQUEOUS EXTRACTS

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Abstract. Our research is focused on the two sub-spices of *Cichorium intybus* L. (ssp. *eu-intybus* and ssp. *pumilum*) collected from Algeria, by phytochemical study including measurement of the total content of phenolic (TPC) and flavonoids (TFC), identifying phenolic profiles and evaluate the antimicrobial activity of aqueous (infusion and decoction) extracts. Four extracts were obtained. Some phenolic acids and flavonoids were identified as ferulic acid, quercetin, and naringenin. The quantification of phenolic compounds shows lower contents for the two components estimated, where the decoction of ssp. *pumilum* are the extract with higher phenolic contents. The antimicrobial activity of the aqueous extracts of the two sub-species was lower than of the standards used (Levofloxacin and nystatin), and the activity was moderate against Gram +, Gram- and fungal strains tested. The decoction of ssp. *pumilum* was the most active extract with zones of inhibitions varying from 6.33 ± 0.58 to 14.67 ± 0.58 mm. *C. albicans* was more resistant. The CMI of the majority of extracts were greater than 25 mg/mL. This study demonstrates that different aqueous extracts from *C. intybus* ssp. *eu-intybus* and *C. intybus* ssp. *pumilum* of aerial parts from Algeria are different in their properties (contents and profiles), and their antimicrobial activity is moderate witch, incite us, to see with others biological activity.

Key words: *Cichorium intybus* L.; aqueous extract; phytochemical study; antimicrobial activity.

INTRODUCTION

The use of various parts of plants in the treatment of diseases has been defined as the term of medicinal plant [11]. Over the world, the medicinal plants, of all known species, constitutes about 25% [30]. The presence of anti-oxidative and antimicrobial agents in plants tissues implies their conservative effect [17].

Also, transmittable diseases caused by microorganisms are a major basis of death-rate and morbidity in humans. Although several antibiotics have been developed to treat these diseases with optimum efficacy, their bad use and misrule, furthermore the microbial mutation has led to the advent of drug-resistant strains. Consequently, over the past decades, antibiotics that are known to recover specific diseases have lost their efficacy [4, 34].

Therefore, it's primordially to turn back to natural products, used in folk medicine as plants to disclose by isolation and characterization the active principle. Our choice was focalized on chicory (*Cichorium intybus* L.) appertains to the Asteraceae family. By Quézel & Santa (1964), there are two subspecies membership to the *Cichorium* genus in Algeria (locally known as "Seriss" and Tilfaf"): *C. intybus* ssp. *eu-intybus* M. and ssp. *pumilum* (Jac.) Ball. [23], the second subspecies differs from the first in its shorter size and slender stem [23]. There are numerous benefits of these plants as a medicinal plants, coffee substitutes and animal forage [29]. Even it is used as a liver tonic, cardiotonic, cholagogue, depurative, hepatomegaly, cephalalgia, inflammations, dyspepsia, colic, and jaundice [12].

To date, no study has considered these two sub-species from Algeria. Thus, the aims of this report are, to screen their phytochemical and characterization of phenolic compounds and evaluate the antimicrobial activity.

MATERIALS AND METHODS

Plant materials

Collected at the flowering stage in June 2018, from Bachdjerah city, Algiers Algeria, specimens of *C. intybus* ssp. *eu-intybus* and ssp. *pumilum* were identified by Pr. H. Abdelkrim, of the National Institute of Agronomy (INA) Algeria. The collected samples consisted of arial parts, which were cleaned and dried in the shade at room temperature in the laboratory and protected from light in the open air for 7 days, then powdered by a grinding machine and conserving until used.

Preparation of extracts

The procedure of extraction was as follows, 15 g of the two subspecies for both infusion (immersing the plant parts in an amount of boiling water) and decoction (the plant parts are boiled in water) ways with 100 mL of distilled water for 20 min. following the method reported by Olayinka and Aiyegoro (2010) [3]. After the extracts were being cold, they were filtered using a Büchner funnel and Whatman No.1 filter paper. Before being dried with the Freeze Dryer instrument (Savant Refrigerated vapour Trap, RV T41404, USA). The water extract was frozen at -20°C for 1 night. The procedure was reputed three times and the crude extract was weighed and the yielded was calculated, and then stored at 4°C until further use. To obtain any concentration for a defined test, we have to mix an adequate powder weight of lyophilized plants and a volume of water.

We obtained four extracts named as follows: Eu1: infusion, Eu2: decoction of *C.* ssp. *eu-intybus* and Pum1: infusion, Pum2: decoction of *C.* ssp. *pumilum* .

Total phenolics contents

The total phenolic contents (TPC) of extracts were measured, using the Folin–Ciocalteu test depicted by Nouasri et al, 2018 [22]. Briefly, a volume of 0.25 mL of the extract (3 tubes), was added to 3.75 mL of

distilled water, followed by 0.25 mL of Folin-Ciocalteu's reagent. Permitted to react for 3 min, then 0.75 mL of 20 % sodium carbonate was added. Tube contents were agitated and heated at 40 °C for 40 min, then, after cooling the absorbance was read at 760 nm. The following equation was obtained from the standard gallic acid graph: Absorbance = 0.1035 gallic acid (µg/mL + 0.1046 (R2:0.98), was used to calculate the concentrations. To express the results as milligrams of gallic acid equivalents per gram of dried weight the unit mg GAE/g.d.w. was used.

Total flavonoid contents

The total flavonoid contents (TFC) of the extracts were assessed by an aluminum chloride method [22]. 1.5 mL of 2% $AlCl_3 \cdot 6H_2O$ dissolved in methanol was added to equal volumes of the diluted extract (3 tubes). The mixture was shaken and after 10 min of incubation at room temperature, the absorbance was read at 440 nm. The following equation Absorbance = 0.2829 quercitin (µg/mL) – 0.1155 (R2:0.99), obtained from the standard quercetin graph, was used to compute the concentrations of flavonoid compounds were calculated. The results were expressed as milligrams of quercitin equivalents per gram of dried weight (mg QE/g.d.w).

HPLC analysis

The different aqueous extracts were put through to the characterization of phenolic compounds by HPLC-UV / DAD analyses, which were carried out with an Agilent 1260 apparatus equipped with a diode array (DAD) UV detector. The analysis was fulfilled in the reverse phase with column C18 (5 µm, 250 × 4.6 mm). The temperature was retained at 22 ± 0.8 °C, and the injection volume selected was 5 µL. The flow rate was fixed at 1 mL/min. The chromatographic conditions consist of phase A: Acetic acid 1 %, phase B: methanol (HPLC grade), with the following gradient: 0 min: 95 % A + 5 % B; 55 min: 5 % A + 95 % B at the end 60 min 95 % A + 5 % B. Detection was effected at 270 nm, 320 nm and 370 nm. The phenolic acids and flavonoids contained in aqueous extracts analyses were recognized by comparing the retention times and the UV spectra obtained by those of the standards used.

Antimicrobial activity

Microbial strains

The different extracts of both plants were individually tested against pathogenic microbes including three Gram-negative bacteria *Pseudomonas aeruginosa* (CIP A22), *Klebsiella pneumonia* E40, *Escherichia coli* (ATCC 10536), and three Gram-positive bacteria *Enterobacter cloacae*, *Staphylococcus aureus* (CIP 7625), *Bacillus subtilis* (ATCC 6633). Four fungi: *Aspergillus flavus* (ATCC 200026), *Penicillium expansum, Fusarium culmorum* (ATCC 36017) and *Candida albicans* (M3). All microorganisms were obtained from The Microbiological Laboratory, Department of Biology, ENS Algiers, Algeria. Bacterial strains were cultured on Muller–Hinton agar (Institute Pasteur, Algeria) for 24h at 37 °C, and fungi were cultivated on Sabouraud dextrose agar (Institute Pasteur, Algeria) for 48h at 25 °C.

Disc diffusion test

The disk diffusion method was used to screen the antimicrobial effect of the extracts [22]. The test strains were put in suspension with sterile saline solution (0.9% NaCl) and the cell density was settled to 0.5 McFarland. Sterile 5.5 mm paper discs, filled with 10 µL of the extracts solutions (100 mg/mL) were placed on the inoculated surface. To enable the diffusion of the extracts from disc to medium without microbial growth, the Petri dishes were stored in the dark at +4 °C for 1 hour; after what incubated for 18-48 h between 25 °C to 37 °C. The diameter of the zones of inhibition around each disc (in millimeters, with the diameter of the paper disc) was taken as a measure of antimicrobial activity. Levofloxacin and nystatin (10 µg/disc) were used as a positive control against bacteria and fungi, respectively [22].

Agar dilution method

The agar dilution method was performed to quantify the minimal inhibition concentration (MIC) of the different extracts [22]. The concentrations between 25–0.097 mg extract/mL medium, were performed with suitable amounts of the extract added aseptically to the sterile medium. The mixture (medium and extract) were mixed forthwith and tipped into Petri plates, and then the plates were inoculated by spotting 3 µL of test strains. After incubation, the presence or absence of growth of microorganisms was taken into consideration. The lowest concentration of the extract needed to inhibit the growth of strains tested was considered the MIC.

Statistical analysis

All experiments were carried out in triplicate. Data were evaluated by one-way analysis of variance (ANOVA) test completed by a T test. Results were expressed as means ± standard deviation (S.D). Differences were considered significant at $p<0.05$.

RESULTS

Yield of extraction

According to the results shown in Figure 1, the decoction mode gives a higher yield of extract ($p<0.05$) compared to infusion mode, and ssp. *pumilum* have yielded more than ssp. *euintybus*.

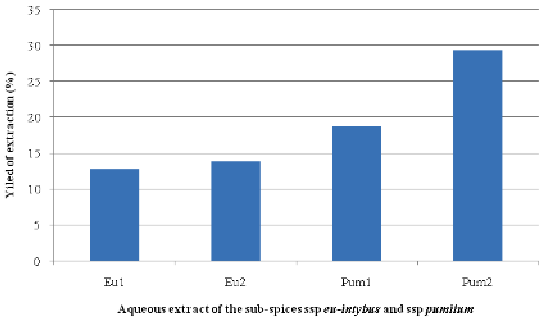

Figure 1. Extraction yields of aqueous extracts from *C. intybus* ssp. *eu-intybus*. All results of yiled of extractions are significantly different ($p < 0.05$).

HPLC analysise

The analyses of the four extracts have shown the presence respectively: as we see in Figure 2 A, B, C and D: there is a similarity in some phenolic compounds between the four different extracts such as ferulic acid, Luteolin 3'-7 diglucoside. Other compounds are specific for infusion as quercetin, and naringenin, for decoction we noted the presence of vanillic acid.

Some others are found in one extract like rosmarinic acid in ssp. *eu-intybus* decoction, caffeic acid and Isorhamnetin present in the infusion of ssp. *pumilum*. Hesperin was not present in the decoction (Pum2), the same case for Luteolin 7 glycoside wish was not found in ssp. *eu-intybus* decoction (EU2).

Quantification of polyphenolic compounds

As reported in Table 1, at all, the results of phenolic compounds are lower, the value of total phenolic ranged from 36.85 ± 1.53 to 40.78 ± 0.37 mg EAG/gms, where Pum1 have the higher content, the descending ranking is as follow Pum1> Pum2 >Eu2>Eu1.

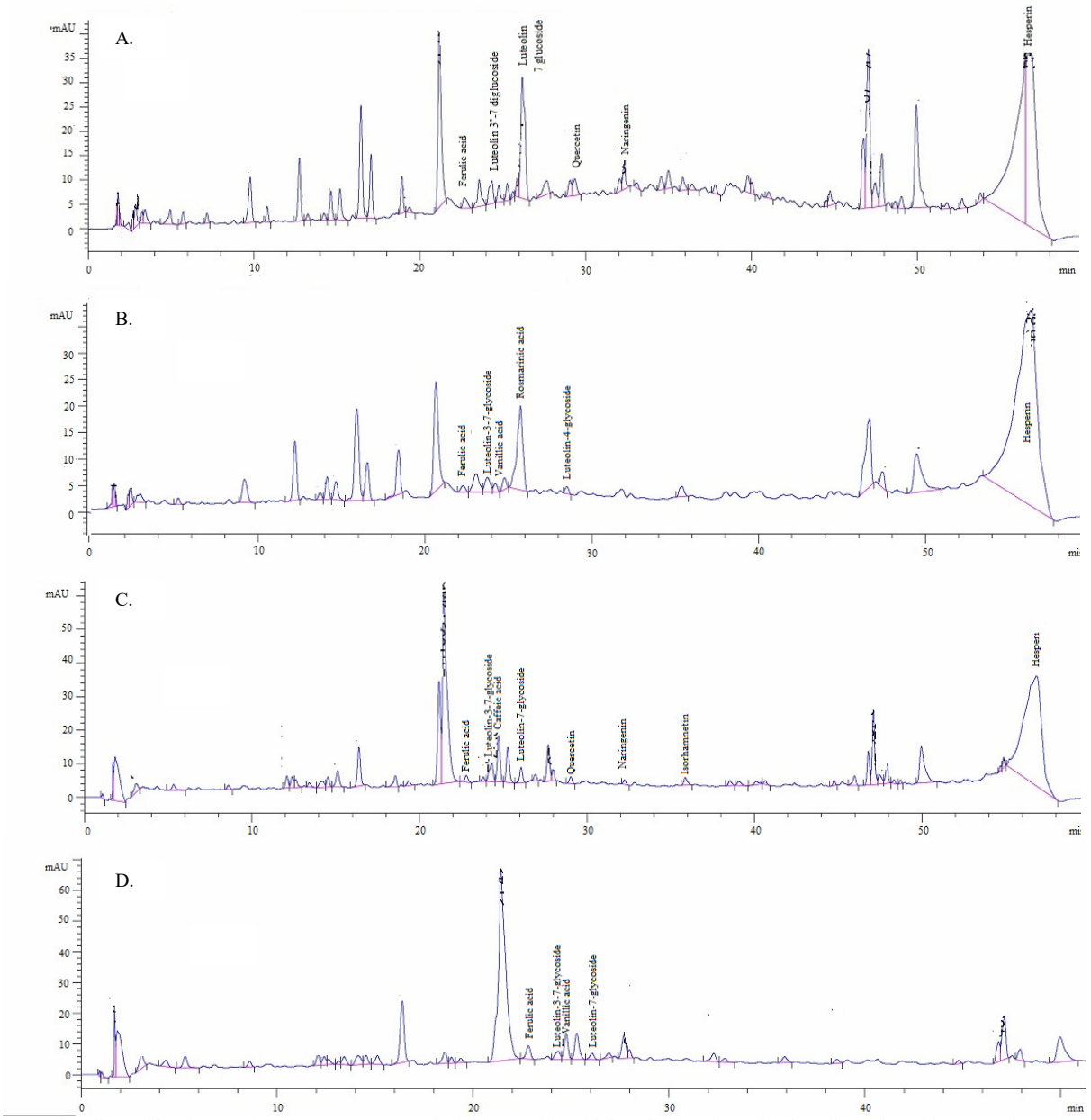

Figure. 2 Phenolic profile of *C. intybus* ssp. *eu-intybus* (A) infusion: Ferulic acid, luteolin-3-7-glycoside, luteolin-7-glycoside, quercetin, naringenin, hesperin. (B) decoction: Ferulic acid, luteolin-3-7-glycoside, vanillic acid, rosmarinic acid, luteolin-4-glycoside, hesperin. And of *C. intybus* ssp. *pumilum* (C) infusion: Ferulic acid, luteolin-3-7-glycoside, caffeic acid, Luteolin-7-glycoside, quercetin, naringenin, isorhamnetin, hesperin. (D) decoction: Ferulic acid, luteolin-3-7-glycoside, vanillic acid, luteolin-7-glycoside; recorded at 320 nm.

Table 1: Total phenolic and flavonoid contents of the different extracts from the two subspecies of *C. intybus*

Extract	EU1	Eu2	Pum 1	Pum 2
Total phenolic compounds (mg EAG/ g.d.w) [ab]	36.85±1.53	39.37±0.70	40.78±0.37	39.98±1.72
Flavonoids (mg EQ/ g.d.w) [ac]	1.28±0.49	0.98±0.05	2.07±0.04	2.15±0.03

[a]Each result is shown as a mean standard deviation (n = 3). [b; c] Different letters indicate significantly different activities ($p < 0.05$). The results of total phenolic and total flavonoid contents are highly significant differences for all data and between columns (p-value < 0.05). No difference in interaction

For the flavonoid content, Pum2 show a significant height level (2.15 ± 0.03 mg EQ/gms), and Eu2 have a low value (0.98 ± 0.05 mg EQ/gms) with descending ranking: Pum2> Pum1 >Eu1>Eu2. It can well be seen that Pum 2 are the extract with high phenolic content compared to the tree other extracts of this study.

Antimicrobial activities

Disc diffusion method and agar dilution methods were used to test the inhibition zone diameter (ID mm) and the minimal inhibition concentration (MIC mg/mL) respectively of infusion and decoction extracts of both subspecies and their results are given in Table 2.

Our finding is that the four extracts of this study presented an antimicrobial activity whether by ID or by MIC inferior to that of the standards levofloxacin or nystatin, and at all the strains tests were resistant to our extracts. It's notably that *Umbelopsis romanniana* was more sensitive especially by MIC value to all extracts of this study.

On the other hand, both *Pseudomonas aeruginous* and *Candida albicans* were the most resistant to all extracts. Eu1 gives an ID of 15.17 ± 0.29 mm, against *B. subtilis* as a height ID mm. The two decoctions extracts were more active against *K. pneumonia* than infusion extracts.

The role of secondary metabolites is a defense mechanism against predation by microorganisms, insects, and herbivores, with other functions, were attributed (odors, pigments etc.), where the ability of plants to composite those elements are limitless, and most of which are phenols or their oxygen-substituted derivatives [22].

In this study, we screened the aqueous extracts of two sub-species of *C. intybus* for their antimicrobial activity. The results of antimicrobial activity were both strains and dose-dependent, with a broad spectrum of inhibition against the tests strains, at different extent, and indicte statistically no significant differences (p-value > 0.05).

DISCUSSION

By consulting the literature, concerning *C. intybus*, we have found that few works have taken into consideration the infusion and the decoction as a mode of extraction, thus, those results will consist of novelty data and the comparison of our results will be restricted to a few references.

Extraction is a substantial procedure for getting bioactive contents from plants. The operation can be influenced by particle size, solid-liquid ratio, solvent type and extraction time employed in the trial, even, temperature, polarity, pH, solubility, and concentration also affect the extraction yields and quality of extracts [11, 27]. Regarding the results of the yield of extraction found in this study, Eray et al. (2020), have obtained 8.2 % in the leaf of *C. intybus* [11], this is the lowest percentage compared to the yield of the two sub-spices of our case. Another study was undertaken by Jasim (2018) of the areal parts reported a yield of 22.75 % [14], this percentage is higher than that of ssp. *eu-intybus* but lower than ssp. *pumilum*. To set out the phenolic profile of the two sub-species infusion decoction extracts (apparently for the first time), by comparing the retention times, and UV-Vis spectra of the samples with the available standards used (we did not refer to data from the literature). From the literature survey (without taking into account the analysis conditions, mode/solvents extract). Tardguno et al. (2018), in their analysis of the alcoholic extract of *C. intybus* from Italy, presents thirteen compounds [32], which were different from the phenolic profile obtained in our case, set apart luteolin-7-glucoside found in the infusion extracts. In the study conducted by Carazone et al. (2013) [6], which resembles the

Table 2. Antimicrobial activity of aqueous extracts from *C.* ssp. *eu-intybus* and ssp. *pumilum*

Stains test	Eu1		Eu2		Pum1		Pum2		Levofloxacin [c]		Nystatin [c]	
	IZD[a]	CMI[b]	IZD[a]	CMI[b]	IZD[a]	CMI[b]	IZD[a]	CMI[b]	IZD[a]	CMI[b]	IZD[a]	CMI[b]
Bacillus subtilis	15.17 ± 0.29	>25	9 ± 1	>25	13.67 ± 1.53	>25	13.67 ± 1.52	>25	36.16 ± 1	0.006	-	-
Staphylococcus aureus	14.23 ± 0.74	12.5	9 ± 1	12.5	6.67 ± 0.58	NT	6.33 ± 0.58	NT	32 ± 1	0.012	-	-
Listeria monocytogenes	9.67 ± 0.58	>25	8.33 ± 0.58	NT	14.27 ± 0.67	>25	6.33 ± 0.58	NT	24.16 ± 0.76	0.024	-	-
Esherichia coli	12 ± 3.46	>25	12.67 ± 2.08	>25	6.67 ± 0.58	NT	13.45 ± 1.36	12.5	29 ± 1	0.024	-	-
Pseudomonas. aeruginosa	NA	NA	NA	NA	NA	NA	NA	NA	24.16 ± 0.76	0.024	-	-
Klebsiella pneumonia	12.13 ± 0.18	>25	14 ± 1.73	12.5	6.33 ± 0.58	NT	14.67 ± 0.58	>25	13.33± 0.57	0.048	-	-
Umbelopsis ramanniana	10 ± 0	<0.39	13.33 ± 1.52	<0.39	9.33 ± 1.15	<0.39	11.33 ± 0.58	<0.39	-	-	Nd	0.94
Aspergillus flavus	9 ± 1	>25	10 ± 1.31	>25	10 ± 10.7	>25	11 ± 2.5	>25	-	-	17 ± 0.5	0.024
Penicillium expansum	13 ± 1.75	>25	11 ± 1.50	>25	9 ± 1	>25	9.33 ± 0.58	>25	-	-	19 ± 1.73	0.003
Candida albicans	NA	NA	NA	NA	NA	NA	NA	NA	-	-	15 ± 1.5	0.12

IZD[a]: Inhibition zone diameter (mm) around the impregnated disks (5mm Ø) and each value is presented as mean ± S.D. (N = 3); CMI[b]: Minimal inhibitory concentration; values are given as mg/mL: [c] Positive controls: levofloxacin for bacteria, nystatin for fungi; NA: no activity; NT : no tested (MIC test, was not realized for the extracts that their ID value under 9mm).

preceding work cited, differs largely from our study and the cafeic acid present in infusion extract of ssp. *pumilum* was the only one comparable, but other works show the presence of different compounds in the phenolic profile of this spice [8, 9, 19, 20].

As known, it's primordial to measure the phenolic compounds content (polyphenol, and flavonoid), due to their medical importance. The present study demonstrated average values of those compounds. Compared with previous data this average rate was higher than those reported by some studies as that of Eray et al. (2020), with aqueous extract of leaf 0.14 ± 0.55 mg EAG/mg and 0.88 ± 2.22 mgEQ/mg (polyphenol and flavonoid respectively) [11]. Also, Kandil et al. (2019) [16], with methanolic and ethanolic extracts from leaves of cichory shows: 0.15 ± 10.25 to 1.16 ± 103.46 GAE ($\mu g \cdot g^{-1}$ d.w.) and 0.036 ± 5.95 to 0.16 ± 5.83 QE ($\mu g \cdot g^{-1}$ d.w.), of polyphenol and flavonoid, respectively. Elgengaihi et al. (2016) with different organic solvents have obtained for methanolic, methylene chloride and petroleum ether respectively 0.00 3 ± 0.34, 0.003 ± 0.26 and 0.003 ± 0.26 mg GAE/mg extract [9]. Epure et al. (2020), with aerial part of *C. intybus* methanolic extract, reported a hight value of flavonoid content of 5.06 ± 0.06 mg RE/g d.w. and low polyphenolic content of 23.94 ± 0.42 mg GAE/g d.w. [10], compared to our findings.

The medium amount got in the current study, is because Water solvents (decoction or infusion) are inefficient solvents for extraction of total phenols from studied plants, for the vast majority of polyphenols are not water-soluble thus, numerous number of residual polyphenols were lost, and only a proper combination of solvents would be able to snatch those compounds [1, 21]. Those agree with our results.

The justification for the swing in the contents is due partially to environmental factors like geographical locations and plant origin affecting the quality and quantity of these molecules and, therefore, their activities [33].

Amongst what this study has revealed, it's the fact that the antimicrobial activity of the infusion and the decoction are lower than organic extracts reported in previous works [7, 24-26, 28]. These extracts were from leaves and stems; therefore, it will be with a lower antimicrobial effect than those from seeds [28], even if it was an aqueous extract [27]. As reported, against *Aspergillus niger*, the water fraction exhibited hopeful activity [26]. That is not the case in this study. At all, and as reported by Janda et al. (2021), in their review study of *Cichorium intybus* L. as a source of compounds having health properties, they signalled the variable antimicrobial effects of this spices organics and aqueous extracts, and has a specific activity towards *Staphylococcus, Streptococcus* and *Escherichia* genres [13]. That constatation doesn't agree with our data, except against *Escherichia coli*.

The differences in the phytochemical constituents of extracts (infused and decocted in this study) are the cause of the dissimilar activity. The different effects against microorganisms are due to the different levels of combinations of bioactive compounds present in different plant parts [15]. Previous work on *C. intybus* [2, 18, 27], attests to the presence of flavonoids, sterols tannins, and this species's richness with triterpenes, coumarins, and alkaloids. These basic constituents of plants have before been indicated to possess antimicrobial activities observed against different strains. As reported in recent studies, phenolics from plant extracts act as antimicrobial agents [5, 18].

Plant-derived phenolics, such as phenolic acids and flavonoids can inhibit the growth and activity of microorganisms. The different structures and chemical compositions of molecules may exhibit various antimicrobial effects, such as inhibition of extracellular enzymes and permeability destabilization of the plasma membrane. The fact which could make plant phenolics effective against drug-resistant pathogens is these mechanisms of action that differ from those of the traditional antibiotics [17].

The analysis by HPLC UV-DAD highlighted the presence of phenolics acids and flavonoids identified in this study reported having antimicrobial activity [7], especially, luteolin and its glycoside, or plants containing those compounds [31].

This study, which is among the first reports, reveals that infusion and decoction extracts, of *C. intybus* ssp. *eu-intybus* and *C. intybus* ssp. *pumilum* (aerial part) from Algeria, have moderate polyphenols contents, also the phenolics acid and flavonoid found in the extracts contribute partially to the antimicrobial activity. Thereby, we propose for those two subspecies, to evaluate others biologicals activities of those extracts such as antioxidant, anti-inflammatory, and isolation of chemical constituents, which may present potential drug ways against different diseases.

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