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Original Research Article

In vitro antioxidant and antimicrobial activities of aerial parts of Algerian *Jurinea humilis* DC (Asteraceae)

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Abstract

Purpose: *To assess the polyphenolic composition of various solvent extracts of Jurinea humilis DC. as well as their in vitro biological effects.*
Methods: *The crude extracts of the aerial parts of Jurinea humilis were obtained by maceration method with dichloromethane (DCM), methanol (MeOH) and ethyl acetate (EtoAC), separately. Folin-Ciocalteu and aluminum chloride procedures were used to quantify the total phenolic and flavonoid contents, respectively, while antioxidant properties were determined by two methods: phosphomolybdenum and DPPH assays. Antimicrobial activity was also evaluated by disc diffusion method.*
Results: *The ethyl acetate extract exhibited the highest amount of total phenolic content (TPC, 169.14 ± 7.22 mg gallic acid equivalent/g sample) and total flavoniod contents (TFC, 104.91±0.22 mg rutin equivalent/g sample). The extract (EtoAC) also showed the strongest antioxidant activity of the three extracts, especially DPPH assay (IC_{50} = 0.16 ± 0.00 mg/mL), as well as demonstrated the highest activity against the pathogenic strains tested.*
Conclusion: *The results suggest that J. humilis DC. is rich in phenolic and flavonoid contents which may be responsible for good antioxidant and antimicrobial properties. Thus, J. humilis may be a promising source of new antioxidant agents and pharmaceuticals.*

Keywords: *Jurinea humilis, Phenolic, Flavonoid, Antioxidant, Antimicrobial*

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INTRODUCTION

Jurinea Cass. (Compositae, tribe Cynareae, subtribe Carduineae) is the largest genus in the family Asteraceae, including about 200 species that are found in diverse regions, especially Central Asia, as well as Iran, Turkey and the Mediterranean basin [1,2]. In the flora of Algeria, the genus *Jurinea* is represented by a single species, *Jurinea humilis* DC. [3].

Although a significant number of plants belonging to *Jurinea* species contain very useful chemical constituents, very few papers have been published on their phytochemical compositions. These chemical studies have revealed the

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presence of several constituents, chief among them, were sesquiterpene lactones and triterpenes [4]. Recently, phytochemical investigation of *J. dolomiaea* roots demonstrated the presence of caffeic acid, apigenin, catechin and rutin [5]. *Jurinea* species have been known for their numerous activities like antimicrobial, antioxidant, anticholinesterase, antilipid peroxidation, anti-toxic and antileishmanial activity [4]. With regard to their quantitative composition (total phenolic and flavonoid contents), there are only three previous reports on *J. dolomiaea* and *J.consanguinea* [5-7].

The objectives of this study were to evaluate the chemical composition (total phenolic and flavonoid contents) of various extracts of the aerial parts of *J. humilis* DC. as well as their *in vitro* antioxidant potentials and antimicrobial activity against different strains of microorganisms.

To best of our knowledge, no previous chemical composition and biological activities have been reported for *Jurinea humulis* DC.

EXPERIMENTAL

Plant material

J. humilis DC. were collected in May 2014 from Boussaada (Algeria), and identified by Professor Hossine Laouar, Laboratory of Natural and Biological Resources Valorization, Department of Biology and Plant Ecology. A voucher specimen was archived in the herbarium of the same laboratory of University Ferhat Abbes, Setif 1 (Voucher no. JH-LH-2014).

Preparation of plant extracts

The collected plant material was cleaned and dried. Each 50 g of dried plant was separately extracted by marceration using 200 mL of the following solvents: dichloromethane (DCM), ethyl acetate (EtoAC) or methanol (MeOH) at room temperature for three days. The filtrates were evaporated to dryness under vacuum using a rotary evaporator at 45 °C.

Determination of antioxidant activity

Total phenolic and flavonoid contents

Total phenolic content was quantified using the Folin–Ciocalteu method [8].The appropriate dilutions (2mg/mL) of 0.2 mL of plant extracts were oxidized with 1 mL Folin–Ciocalteu reagent. After, 2 mL of 7.5% Na_2CO_3 were added. The mixture was then stirred in 7 mL of distilled water, and incubated for 2 h in the dark at room temperature. The absorbance value was measured at 765 nm using a UV/Visible spectrophotometer (Thermo Electron Corporation evolution 100). The total phenolic content was presented as gallic acid equivalent per gram of dry extract (GAE mg/g extract).

Total flavonoid content of extract samples was measured also [9]. Briefly, a mixture of equal volumes (1 mL) of 2 mg/mL extract and 2 % methanolic aluminium chloride ($AlCl_3$) solution was prepared and incubated for 10 min. After, the absorbance was read at 415 nm. Methanol was used as blank. Results were presented as rutin equivalent per gram of dry extract (mg RE/g of extract).

Total antioxidant capacity

Total antioxidant capacity of samples was determined according to phosphomolybdate method of Saeed et al. with slight modification [10].In brief, 3 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were added to 0.3 mL of each extract (2 mg/mL). The absorbance was measured against blank at 695 nm using a UV/visible spectrophotometer (Thermo Electron Corporation evolution 100), after incubation for 90 min at 95 °C. Antioxidant capacity of the extracts was expressed as ascorbic acid (mg AE/g extract) equivalent.

Free radical scavenging capacity (DPPH, 2, 2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity of extracts was investigated by the method of Kirby and Schmidt with a partial modification [11]. Briefly, each 0.5 mL of diluted solutions (0.2 - 1 mg/mL) of extracts in methanol was mixed with 3 mL of DPPH methanolic solution (6.10^{-5} M). The mixture was allowed to react in the dark for 30 min at room temperature.

Absorbance was measured at 517 nm using a UV/visible spectrophotometer (Thermo Electron Corporation evolution 100). The control was prepared as above without any extract. Inhibition (H) was determined as in Eq 1.

$$H(\%) = \{(A_0 - A_1)/A_0\}100 \; \ldots\ldots\ldots. \; (1)$$

where A_0 = absorbance of the control, A_1 = sample/absorbance of standard), BHT was used as standard. The results are expressed as median inhibitory concentration (IC_{50}).

Determination of antimicrobial activity

Microbial strains

The antimicrobial activity of all extracts was tested against a total of 13 microorganisms. These included fish pathogenic strains, one Gram positive bacteria: *Yersinia ruckeri*, and two Gram negative bacteria: *Lactococcus garvieae*, *Vibrio anguillarum* (A4 strains, obtained from different companies), added to clinical and food-borne pathogenic microorganisms. Gram positive bacteria: *Bacillus cereus* (RSKK 86), *Micrococcus luteus* (NRRL B-4375), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 7644) and Gram negative bacteria: *Yersinia enterocolitica* (NCTC 11175), *Escherichia coli* (O157: H7), *Escherichia coli* (ATCC 11229), *Escherichia coli* (ATCC 35218). Two yeasts: *Candida albicans* (ATCC 10231) and *Candida glabrata* (RSKK 04019) were also used in this study.

Bacterial strains were cultured on plate tryptic Soy Agar (TSA) and nutrient agar (NA). Meanwhile yeasts were grown in YPD medium. Bacterial cultures were incubated for 24 h at 37 °C except fish pathogenic bacteria were incubated at 25 °C. Yeasts were incubated for 48 hat 30 °C.

Determination of antimicrobial effect

The disc diffusion method was employed to determine the *in vitro* antimicrobial action of samples [12,13]. Firstly, the plates were spread by culture suspensions and adjusted to 0.5 McFarland. Thereafter, filter paper discs (6 mm in diameter), saturated with 20 µL of prepared extract (2 mg extract/disc) were placed on the inoculated plates and then stored in a refrigerator during (2 h), in order to ensure prediffusion of each extracts into the agar. At last plates were incubated at appropriate temperatures (37 ºC for 24 h for bacteria, at 25 °C for fish pathogenic bacteria and at 30 ºC for 48 h for yeast strains).
Ampicillin (Amp, 10 µg/disc), gentamicin (CN, 10 µg/disc) were chosen as standard antibacterial while fluconazole (FCA, 25 µg/disc) was chosen as standard antifungal. Negative controls were performed with paper discs impregnated with solvents. The measuring zones of growth inhibition around the disc in millimeters (mm) were applied to evaluate the antimicrobial activity. All assays were carried out in triplicate.

Determination of minimal bactericidal (MBC) or fungicidal concentration (MFC)

To determine the MBC and MFC values of the extracts two fold dilutions were followed [13,14]. The strains that showed an inhibition zone in our assay were subjected to determine their MBC and MFC values. Therefore, each of extracts: the dichloromethane (DCM), ethyl acetate (EtoAC) and methanol (MeOH) were dissolved in test tubes, at the initial concentration of 60.00 mg/mL, and then varying dilutions were obtained even to achieve final concentration of 0.94 mg/mL. At first, the microorganisms were inoculated during 12h and the resulting cultures suspensions were adjusted to 0.5 McFarland. A volume of 2.5 µL of the tested microorganism was introduced to each tube and further completed by using 100 µL of the respective medium.

Positive controls were prepared by mixing 2.5 µLof inoculum and 100 µL of growths medium in tubes. On the other hand, negative controls were performed with tubes wich contains 2.5 µL of extract and 100 µL of growth medium. Thereafter, the content of prepared tubes was incubated for 24 h. At the end, a fixed volume of 5 µL of the sample was taken from each tube in order to inoculating on solid growth medium. The concentration that killed the microorganism in this medium was taken as MBC or MFC of extract.

Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. Differences were considered significant when $p < 0.05$. Analyses were carried out using Statistica version 7.0 (StatSoft, Inc, Tulsa, OK, USA).

RESULTS

Total phenolic and flavonoid contents

The phenolic and flavonoid contents of *J. humulis* extracts varied significantly different ($p<0.05$), with a marked predominance of phenolic contents in all extracts. Furthermore, the ethyl acetate extract exhibited the highest rates of polyphenolic compounds (Table 1). The results of Table 1 indicated that the highest concentration of total phenolic was of (169.14 ± 7.22 mg GAE/g dry weight) for the ethyl acetate extract, whereas the lowest content value was obtained from the dichloromethane extract (59.04 ± 9.26 mg GAE/g dry weight). Additionally, the highest level of total flavonoid content was of (104.91 ± 0.22 mg RE/g dry weight) for ethyl acetate extract when compared to dichloromethane and methanol extracts (56.67 ±

0.14 and 49.54 ± 1.94 mg RE/g dry weight), respectively.

Table 1: Total phenolic and flavonoid contents of *J. humulis* extracts

Extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)
Dichloromethane	59.04±9.26^{a}	56.67±0.14^{b}
Methanol	124.4±4.24^{b}	49.54±1.94^{a}
Ethyl acetate	169.14±7.22^{c}	104.91±0.22^{c}

Note: *Data are mean± standard deviation (SD, n =3). Values in the same column followed by a different superscript letter ($^{a-c}$) are significantly different (p < 0.05)*

Total antioxidant capacity and free radical scavenging activities (DPPH)

Table 2: Total antioxidant activity and free radical scavenging capacity of *J. humilis* extracts

Extract	TAC* (mg AAE/g)	IC_{50}** (mg/mL)
Dichloromethane	290.23±9.16^{b}	0.60±0.01^{c}
Methanol	182.23±12.51^{a}	0.36±0.03^{b}
Ethyl acetate	337.62±11.42^{c}	0.16±0.00^{a}
BHT	-	0.05±0.01^{a}

**Total antioxidant capacity (TAC) expressed as ascorbic acid equivalent (mg AAE/g extract). ** Results of DPPH assay; Butylated hydroxytoluene (BHT) was used as positive control. Data are mean ± standard deviation (SD, n =3). Values in the same column followed by a different superscript letter ($^{a-c}$) are significantly different (p< 0.05)*

Total antioxidant capacity of studied *Jurinea* extracts was summarized in the Table 2. All extracts were recorded important antioxidant capacity varying between 182.23 and 337.62 mg AAE/g. The greatest antioxidant efficacy (337.62 ± 11.42 mg AAE/g) was noticed with ethyl acetate (EtoAC) extract, followed by dichloromethane (DCM) and methanol (MeOH) extracts (290.23 ± 9.16 and 182.62 ± 12.51 mg AAE/g, respectively).

J. humilis extracts showed different DPPH free radical scavenging activity ($p < 0.05$, Table 2). A higher radical scavenging activity was produced by the ethyl acetate extract (IC_{50} = 0.16 ± 0.00 mg/mL); this was equivalent to the standard, BHT ($p < 0.05$).

Antimicrobial activity

As shown in Table 3 and Table 4, the inhibition zone diameter for strains were in the ranges 7.48-22.55 mm. Except for the methanol (MeOH) extract, antibacterial effects against the fish pathogens was better than clinical and food-borne pathogens. Moreover, the findings revealed that the antibacterial behavior of extracts had a wide inhibition spectrum (p<0.05) for both Gram-negative and Gram-positive bacteria.

As seen in Table 3, maximum *in vitro* inhibition of tested bacteria was obtained in the ethyl acetate (EtoAC) extract which had inhibition zone diameter of 22.55 mm against *V. anguillarum*A4 followed by 17.71 mm against *Y. ruckeri*, and 16.17 mm against *L. garvieae*.

Overall, the ethyl acetate (EtoAC) and dichloromethane (DCM) extracts demonstrated the highest antibacterial efficiency against fish pathogenic microorganisms with marked activity against Gram-negative (-) bacteria, compared with gentamicin (CN, 10 µg/disc).

In the case of clinical and food-borne pathogens (Table 4), different extracts of *J.humilis* exhibited antimicrobial activity. The extract of ethyl acetate (EtoAC) showed the highest inhibitory effect for all microorganisms, especially against *S. aureus* (ATCC 25923) which recorded significant

Table 3: Antibacterial activity of *J. humilis* extracts against fish pathogens

Test microorganism	Inhibition zone diameter* (mm)			Antibiotics Inhibition zone diameter* (mm)	
	MeOH-E (2 mg/disc)	DCM-E (2 mg/disc)	EtoAC-E (2 mg/disc)	Amp	CN
Gram +					
L. garvieae	8.77±0.76^{a}	14.39±1.06^{c}	16.17±0.81^{d}	30.17±2.39^{e}	11.77±1.48^{b}
Gram -					
V.anguillarum A4	9.46±1.25^{a}	15.79±0.25^{c}	22.55±0.99^{d}	29.72±1.51^{e}	10.76±0.66^{b}
Y. ruckeri	9.36±0.41^{a}	14.84±3.51^{b}	17.71±0.69^{d}	29.50±0.71^{e}	17.16±0.62^{c}

**: Diameter of the inhibition zone including disc diameter; Amp: ampicillin; CN: gentamicin; MeOH-E: methanol extract; DCM-E: dichloromethane extract; EtoAC-E: ethyl acetate extract. Values are reported as means ± standard deviation (SD; n=3). Values in the same line followed by a different superscript letter ($^{a-e}$) are significantly different (p< 0.05)*

Table 4: Antimicrobial activity of *J. humilis* extracts against clinical and food-borne pathogens

Test microorganism	Inhibition zone diameter∗ (mm)			Antibiotic inhibition zone diameter* (mm)		
	MeOH-E (2mg/disc)	DCM-E (2mg/disc)	EtoAC-E (2mg/disc)	Amp	CN	FCA
Gram +						
B.cereus	10.46±0.57[a]	11.43±0.40[b]	11.70±0.61[c]	37.23±0.21[e]	13.89±0.68[d]	-**
M. luteus	8.80±0.58[a]	10.06±0.44[b]	11.02±0.49[c]	26.25±0.26[e]	13.85±0.21[d]	-
S. aureus	11.17±0.59[a]	13.23±0.39[b]	15.23±0.27[d]	24.93±1.34[e]	14.65±0.19[c]	-
L. monocytogenes	7.48±0.31[a]	10.82±0.41[c]	10.48±0.59[b]	26.75±0.66[e]	14.77±0.05[d]	-
Gram -						
Y. enterocolitica	8.36±0.15[a]	11.09±0.92[b]	11.82±0.39[c]	25.25±0.29[e]	22.13±0.07[d]	-
E. coli O157:H7	8.48±0.26[a]	11.09±0.52[b]	11.39±0.71[c]	26.54±0.89[e]	12.27±1.25[d]	-
E. coli ATCC 11229	9.25±0.47[a]	9.55±0.30[b]	9.82±0.36[c]	24.72±0.15[e]	12.32±0.61[d]	-
*E.coli*ATCC 35218	9.19±0.68[a]	9.40±0.63[b]	11.00±0.32[c]	24.03±0.42[e]	13.73±0.49[d]	-
Yeasts						
C. albicans	9.51±0.23[a]	10.68±0.11[b]	11.77±0.63[c]	-	-	21.85±1.76[d]
C. glabrata	10.91±0.28[a]	11.99±1.58[b]	14.20±2.05[c]	-	-	25.06±1.92[d]

**Diameter of the inhibition zone including disc diameter. **Indicates no antimicrobial activity; Amp: ampicillin; CN: gentamicin; FCA: fluconazole; MeOH-E: methanol extract; DCM-E: dichloromethane extract; EtoAC-E: ethyl acetate extract. Values are reported as mean ± standard deviation (SD, n = 3). Values in the same line followed by a different superscript letter (a-e) are significantly different (p< 0.05)*

Table 5: MBC and MFC (mg/mL) of *J. humilis* extracts against fish, clinical and food-borne pathogens

Test microorganism	MBC/MFC (mg/mL)		
	MeOH-E	***DCM-E***	***EtoAC-E***
Fish pathogens			
Gram+			
L. garvieae	60.00	15.00	15.00
Gram-			
V. anguillarum A4	60.00	30.00	1.88
Y. ruckeri	30.00	30.00	15.00
Clinical and food-borne pathogens			
Gram+			
B. cereus RSKK 863	7.50	7.50	7.50
M. luteus NRRL B-4375	30.00	15.00	30.00
S. aureus ATCC 25923	15.00	1.88	1.88
L. monocytogenes ATCC 7644	60.00	30.00	30.00
Gram-			
Y. enterocolitica NCTC 11175	15.00	15.00	7.50
E. coli O157:H7	30.00	15.00	3.75
E. coli ATCC 11229	30.00	15.00	7.50
E. coli ATCC 35218	30.00	15.00	3.75
Yeasts			
C. albicans ATCC 10231	30.00	15.00	15.00
C. glabrata RSKK 04019	30.00	15.00	15.00

***Note:** MBC: minimal bactericidal concentration; MFC: minimal fungicidal concentration*

inhibition zone of 15.23 mm compared with gentamicin (CN, 10 µg/disc).

With regard to the results of antifungal activity (Table 4), the extracts showed moderate activity, and exhibited almost the same activity against *C. albicans* (ATCC 10231) with inhibition zone diameter ranging from 9.51 to 11.77 mm. Furthermore, ethyl acetate (EtoAC) extract showed higher inhibition zone diameter of 14.20 mm against *C. glabrata* (RSKK 04019) while dichloromethane (DCM) and methanol (MeOH) extracts recorded inhibition zones diameters of 11.99 and 10.91 mm, respectively.

The MBC values (Table 5) of extracts against fish pathogens were ranged between 1.88 and 60.00 mg/mL. Ethyl acetate extract showed lower MBC than the other extracts. The lowest value (1.88 mg/mL) was detected for *V. anguillarum* (strain A4).

The MBC and MFC values against clinical and food-borne pathogens (Table 5) were in accord with the results of Table 4, and MBC of 1.88 mg/mL was estimated against *S. aureus* (ATCC 25923) followed by3.75mg/mL against *E. coli*(O157:H7) and *E. coli*(ATCC 35218).

The MFC values of the extracts varied from 15 to 30 mg/mL. In the same test, the MFC values of the ethyl acetate (EtoAC) and dichloromethane (DCM) extracts was 15 mg/mL for tested fungi. With reference to the antifungal activity of fluconazole (FCA, 25 µg/disc), the antifungal effect of the extracts was good, but their inhibitory potency was weak. However, Candida strains showed resistance against many antifungal drugs.

DISCUSSION

Phenolic compounds including flavonoids form the large group of secondary metabolites, exhibits various structures that make them major contributors to the overall pharmacological potentials like antioxidant and antimicrobial activities of many plants [15-18]. In this context, it was reasonable to assess the total phenolic (TPC) and flavonoid (TFC) contents of the extracts as well as their antioxidant and antimicrobial activities. All the extracts showed different levels of polyphenolic compounds (TPC and TFC).In addition, ethyl acetate proved to be the best solvent in extracting TPC and TFC in maximum quantity. These results are similar to those reported in the literature for *Jurinea dolomiaea* [4-6]. In contrast, *J. consanguinea* is not rich in polyphenolic compounds [7].
On the other hand, the stronger total antioxidant efficacy and their DPPH radical scavenging ability were highest in ethyl acetate extract of *J. humilis*, which contained high levels of phenolics and flavonoids. These results are similar to those of previous studies on *J. dolomiaea* [4-6]. The present work was consistent with reported findings which confirmed that polyphenols and flavonoids are responsible for most of the antioxidant capacity of the extracts [17-20].

Several studies have examined the structure-activity relationship of flavonoids. It is well accepted that the antioxidant behavior of flavonoids is clearly influenced by the number and position of hydroxyl groups on their structure. Usually, polyphenols with a high number of hydroxyl groups have the highest antioxidant activity [19].

The results of the antibacterial activity of *J. humilis* extracts are in accordance with previous research on *J. dolomiaea*, *J. consanguinea* [6,7] and *J. ancyrensis* [21]. Similarly, our species possess a marked antibacterial activity against *Staphylococcus aureus*. In contrast, the antifungal activity of *J. humilis* extracts was not significant when compared with that of *J. ancyrensis* extracts [21].

The strong antimicrobial activity of ethyl acetate extract might be due to its phenolic and flavonoid contents [19]. These classes of secondary metabolites are useful as antimicrobial agents, and are capable of inhibiting the growth of microorganisms depending on their concentration [22,23].

CONCLUSION

The antioxidant and antimicrobial activities of extracts of *Jurinea humilis* DC have been reported in this study. The findings reveal that the ethyl acetate extract of *J. humilis* has higher total phenolic and flavonoid contents, as well as exhibit the highest antioxidant and antimicrobial activities of the extracts studied. Taking into consideration the results of antimicrobial activity of *J. humilis* extracts against fish, clinical and food-borne pathogens, it can be deduced that the ethyl acetate extract of *J. humilis* may be a new source for natural antibacterial agent against *Vibrio anguillarum* and *Staphylococcus aureus*. Thus, *J. humilis* DC. merits further phytochemical investigation to elucidate its chemical composition and molecular structure, and also to determine in depth its other biological activities.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Authors' contributions

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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