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Phytochemical analysis and biological activities of *Hertia cheirifolia* L. roots extracts

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ABSTRACT

Objective: To test the antioxidant, antimicrobial and α-glucosidase inhibitory activities of the roots extracts from *Hertia cheirifolia* (*H. cheirifolia*) L.

Methods: Total phenolics and total flavonoids content of the different extracts were determined by colorimetric methods and reverse phase high-performance liquid chromatography (RP-HPLC) was performed to identify various chemical components. The different extracts were evaluated for antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylenebenzothiozoline-6-sulfonic acid ($ABTS^{\bullet+}$) and β-carotene bleaching tests and α-glucosidase inhibitory properties. The antimicrobial activity was carried out *in vitro* by the broth dilution method.

Results: *Trans*-cinnamic acid, rutin hydrate, naringin and quercetin were the main compounds of the ethyl acetate extract from *H. cheirifolia* L. This extract has significant scavenging activity to decrease free radicals especially for DPPH and ABTS radicals. As well as, the ethyl acetate extract exhibited an antimicrobial property against bacterial strains. *Bacillus licheniformis* and *Salmonella enterica* were the most sensitive strains with minimum inhibitory concentration values of 0.156 mg/mL.

Conclusion: The ethyl acetate extract was found to be selectively antioxidant and antimicrobial.

1. Introduction

Natural products, especially those of vegetable origin, have always been an important source of therapeutic agents. About 25%–30% of drugs available for the treatment of diseases are derived from natural products [1].

Due to the advance of synthetic chemistry, research on natural products in the pharmaceutical industry is limited. However, recent data from this industry show that these products represent an extremely valuable source for the production of new chemical molecules, because they represent privileged structures chosen by evolutionary mechanisms over a period of millions of years [2].

Many studies carried out in the field of ethnopharmacology show that plants used in traditional medicine and which have been tested are often efficient plants in pharmacological models. Thereby, the medicinal plants and natural extracts have been considered as alternative therapy against various diseases [3].

In this context, belonging to the Asteraceae family, *Hertia cheirifolia* (*H. cheirifolia*) L. is known in Tunisia as medicinal plant and used traditionally to reduce hyperglycemia [4]. Its extracts have demonstrated spasmolytic, anti-inflammatory, acaricidal, antibacterial, antioxidant activities and α-glucosidase inhibition [5–8].

The objective of this study was to perform the preliminary phytochemical screening and to determine the total phenolic and flavonoid contents, as well as antioxidant, antimicrobial activities and α-glucosidase inhibition of *H. cheirifolia* roots extracts.

2. Materials and methods

2.1. Plant material

H. cheirifolia L. was harvested at the flowering stage from the soils of Thala in February 2012. The roots of this plant were reduced to coarse powder and extracted by maceration in the methanol [MeOH/H_2O 80:20 (v/v)] for 72 h at room

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temperature. The hydro-methanolic was further subjected to a successive extraction using petroleum ether, ethyl acetate and butanol to yield dried fractions.

2.2. Qualitative analyzes of H. cheirifolia roots extracts

2.2.1. Estimation of saponins

According to Mir *et al.* [9], saponins content of *H. cheirifolia* was estimated by dissolved 5 mg of extract in 10 mL of hot distilled water (50 °C). The height of the persistent foam was measured in cm.

2.2.2. Estimation of polyphenols

The technique used consists of dissolving 5 mg of each extract in 1 mL of distilled water and then adding a few drops of ferric chloride 2%. The green color indicates the presence of polyphenols [10].

2.2.3. Estimation of flavonoids

Five mg of each extract is dissolved in 3 mL of methanol and then treated with a drop of concentrated hydrochloric acid and 0.5 g of magnesium chips. Three minutes later, a pink or red coloration indicates the presence of flavonoids [10].

2.2.4. Estimation of steroids and triterpenes

Steroids and triterpenes determination was done according to Alebiosu and Yusuf [11]. Five mg of each extract was dissolved in 5 mL of distilled water, 5 mL of acetic anhydride and a few drops of concentrated sulfuric acid. Thirty minutes later, steroids give a red color with this reaction, whereas the appearance of a green color indicates the presence of triterpenes.

2.3. Quantitative analyzes of H. cheirifolia roots extracts

2.3.1. Total phenolic content

The total phenolic amount was determined by using Folin–Ciocalteu reagent [12]. A total of 100 µL of extract was dissolved with 750 µL of Folin–Ciocalteu reagent and 750 µL of saturated sodium carbonate solution. After 90 min, the absorbance was recorded at 765 nm with an UV–vis spectrometer.

2.3.2. Total flavonoids content

The content of total flavonoids was determined using the aluminum chloride ($AlCl_3$) [13]. A volume of 1.5 mL (1 mg/mL) of extract was added to an equal volume of a 2% $AlCl_3 \cdot 6H_2O$ solution. The mixture was vigorously shaken, and the absorbance was recorded at 367 nm after 10 min of incubation with an UV–vis spectrometer.

2.4. In-vitro antioxidant activities

2.4.1. The radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH•)

According to Hamdi *et al.* [14], 500 µL of each extract concentration was mixed with 500 µL of DPPH• methanolic solution. Three minutes later, the absorbance of the resulting solution was measured at 520 nm with a spectrophotometer. All measurements were performed in triplicate.

2.4.2. The radical cation scavenging activity of 2,2-azino-bis-3-ethylenebenzothiozoline-6-sulfonic acid ($ABTS^{\bullet+}$)

According to Lv *et al.* [15], 900 µL of the solution of ABTS was added to 100 µL of the extracts dissolved in ethanol. Twenty minutes later, the absorbance values were read at 734 nm. All measurements were performed in triplicate.

2.4.3. Inhibition of β-carotene bleaching test

Fifty milliliters of distilled water were added to the mixture of 2 mL of β-carotene solution, 20 µL of linoleic acid and 200 µL of Tween-20. Then, 5 mL of this resulting solution were added to 500 µL of extracts and incubated in a water bath at 50 °C for 60 min. The absorption of the reaction was read at 470 nm [16].

2.5. Determination of minimum inhibitory (MIC), minimum bactericidal (MBC) and minimum fungicidal (MFC) concentrations

The MIC of the samples was determined by microdilution on a plate divided into 96 wells, it was defined as the lowest concentration of sample that inhibited the microbial growth after incubation at 37 °C for 18–24 h [17]. The MBC and MFC were determined by subculture on blood agar at 37 °C for 24 h [14].

2.6. α-Glucosidase inhibition test

The α-glucosidase reaction mixture, contained 2.5 mmol/L 4-*p*-nitrophenyl-α-D-glucopyranoside, 250 µL of extract and 0.3 U/mL α-glucosidase in phosphate buffer pH 6.9, was incubated in a water bath at 37 °C for 15 min. Absorbance of the resulting *p*-nitrophenol was determined at 405 nm and was considered directly proportional to the activity of the enzyme [18].

2.7. Analysis of phenolic compounds by analytical RP-HPLC/UV

The separation of phenolic compounds was performed with an Agilent 1100 series HPLC system equipped with on-line degasser (G1322A), a quaternary pump (G 1311A), a thermostatic autosampler (G 1313A), a column heater (G 1316A) and a diode array detector (G 1315A). The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% formic acid (solvent B). The flow rate was kept at 0.7 mL/min. The gradient program was as follows: 35% A/65% B (0–6 min), 60% A/40% B (6–9 min), 80% A/20% B (9–14 min), 100% A (14–25 min), 35% A/65% B (25–30 min). The injection volume was 20 µL and peaks were monitored at 280 nm. Peaks were identified by congruent retention times compared with standards.

2.8. Statistical analysis

Results are given as mean ± SEM. Data were subjected to one-way ANOVA, and Duncan's multiple range tests was used to compare means. Statistical analyses were performed with the SPSS statistical software program (SPSS v.16). Statistical significance was set at $P < 0.05$.

3. Results

The results obtained from the phytochemical tests are presented in Table 1. This preliminary phytochemical screening revealed the presence or absence of these secondary metabolites. The examination of this table showed the presence of saponosides, flavonoids, polyphenols, sterols and triterpenes in the various extracts with varying intensities. In fact, the foaming character of the saponins is very important in the hydro-methanolic and butanolic extracts. The results showed that the detection of flavonoids in hydro-methanolic, ethyl acetate and butanolic extracts was confirmed by the appearance of a pink color in the reaction medium. Thereby, the positive test of the triterpenes indicated their presence in the extracts with an appearance of a green color. The presence of polyphenols was noted essentially in the ethyl acetate extract.

Table 1
Phytochemical screening of *H. cheirifolia* roots extracts.

Extracts	Saponins	Polyphenols	Flavonoids	Triterpenes
Hydro-methanolic	+++	++	++	+++
Petroleum ether	–	–	–	+++
Ethyl acetate	–	++	+++	+++
Butanolic	+++	++	++	+++

+++: relatively a strong presence; ++: relatively moderate presence; +: relatively slight presence; –: Absence.

Table 2
Quantitative determination of phenolic compounds in *H. cheirifolia* roots extracts.

Extracts	Phenols (mg GAE/g dry extract)	Flavonoids (mg QE/g dry extract)
Hydro-methanolic	5.39 ± 0.20^{b}	7.69 ± 1.20^{b}
Petroleum ether	0.17 ± 0.01^{a}	0.23 ± 0.03^{a}
Ethyl acetate	9.33 ± 1.00^{d}	69.35 ± 2.00^{d}
Butanolic	7.45 ± 0.07^{c}	14.74 ± 1.60^{c}

GAE: gallic acid equivalents; QE: quercetin equivalents; The different letters indicate a significant difference between the extract ($P < 0.05$).

Table 3
Antioxidant activity of *H. cheirifolia* roots extracts (mg/mL).

Extract	IC_{50}		
	DPPH	ABTS	β-carotene/ linoleic acid
Hydro-methanolic	0.147 ± 0.010^{d}	0.310 ± 0.020^{d}	0.160 ± 0.010^{b}
Petroleum ether	0.380 ± 0.020^{e}	0.550 ± 0.020^{e}	0.481 ± 0.010^{e}
Ethyl acetate	0.029 ± 0.002^{b}	0.120 ± 0.010^{b}	0.230 ± 0.010^{c}
Butanolic	0.098 ± 0.006^{c}	0.240 ± 0.010^{c}	0.344 ± 0.020^{d}
Butylated hydroxytoluene	0.018 ± 0.001^{a}	0.050 ± 0.001^{a}	0.040 ± 0.001^{a}

Values are expressed as mean ± SEM ($n = 3$). The different letters indicate a significant difference between the extract ($P < 0.05$). IC_{50}: the concentration at which 50% is inhibited. DPPH: 2,2-diphenyl-1-picrylhydrazyl. ABTS: 2,2-azino-bis-3-ethylenebenzothiozoline-6-sulfonic acid.

The results in Table 2 showed that the ethyl acetate extract contains the highest content of polyphenolic compounds [(9.33 ± 1.00) mg GAE/g]. The total phenolics content in butanolic and hydro-methanolic extracts were (7.45 ± 0.07) GAE/g and (5.39 ± 0.20) mg GAE/g, respectively. But there were traces of phenolic compounds in the petroleum ether extract [(0.17 ± 0.01) mg GAE/g]. Similarly for the total content of flavonoids, the ethyl acetate extract was relatively the rich extract in flavonoids [(69.35 ± 2.00) mg QE/g].

Table 3 showed the inhibited concentration (IC_{50}) values of the various extracts. It should be noted that the lowest value of IC_{50} indicates the strongest activity against free radicals. The results obtained showed that the ethyl acetate extract was the most active extract. Indeed, it revealed an interesting activity of trapping of the radical DPPH• and $ABTS^{\bullet+}$ with IC_{50} values of (0.029 ± 0.002) mg/mL and (0.120 ± 0.010) mg/mL, respectively. While, an outstanding activity ($P < 0.05$) of the hydro-methanolic extract in the inhibition of the β-carotene bleaching was observed [IC_{50} = (0.160 ± 0.010) mg/mL].

The different *H. cheirifolia* extracts showed various degrees of inhibition, evaluated as MIC and MBC against the tested strains. The results were compared to gentamicin used as positive control for bacteria (Table 4). The ethyl acetate extract had a significant inhibitory effect on $Gram^{+}$ and $Gram^{-}$ bacterial strains, this effect was highlighted by the measure of MIC which reached its minimum value against *Bacillus licheniformis* and *Salmonella enterica* (MIC/MBC = 0.156/0.312 mg/mL). However, *Salmonella typhimurium* was more resistant with MIC of 1.250 mg/mL.

As shown in Table 5, the fungal strains tested were resistant to the various extracts. However, the hydro-methanolic extract exhibited a fungicidal effect on *Candida albicans* (MIC/MFC = 0.625/1.250 mg/mL). Amphotericin B was the positive control.

A comparison of the IC_{50} of the various extracts with the reference product [IC_{50} = (0.280 ± 0.010) mg/mL] showed that the butanolic extract was moderately active with IC_{50} of

Table 4
Antibacterial activity of roots extracts from *H. cheirifolia* (mg/mL).

Strains	Hydro-methanolic extract		Petroleum ether extract		Ethyl acetate extract		Butanolic extract		Gentamicin
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MBC
Staphylococcus aureus	1.250	5.000	1.250	>5.000	0.312	0.625	1.250	2.500	0.031
Bacillus subtilis	2.500	5.000	na	na	0.312	1.250	na	na	0.062
Bacillus licheniformis	1.250	2.500	2.500	>5.000	0.156	0.312	2.500	5.000	0.031
Esherichia coli	1.250	2.500	0.625	2.500	0.625	1.250	2.500	5.000	0.125
Pseudomonas aeruginosa	2.500	5.000	2.500	>5.000	0.625	1.250	2.500	>5.000	0.500
Salmonella enterica	1.250	2.500	1.250	>5.000	0.156	0.312	2.500	5.000	0.125
Salmonella typhimirium	2.500	5.000	na	na	1.250	2.500	2.500	>5.000	0.250

na: not active; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

Table 5
Anticandidal activity of roots extracts from *H. cheirifolia* (MIC/MFC in mg/mL).

Extracts	*Candida albicans*		*Candida glabrata*		*Candida krusei*		*Candida parapsilosis*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Hydro-methanolic extract	0.625	1.250	2.500	5.000	1.250	2.500	2.500	5.000
Petroleum ether extract	1.250	>5.000	na	na	2.500	>5.000	na	na
Ethyl acetate extract	5.000	>5.000	5.000	>5.000	na	na	5.000	5.000
Butanolic extract	5.000	>5.000	na	na	2.500	5.000	5.000	>5.000
Amphotericin B	–	0.050	–	0.050	–	0.050	–	0.050

na: not active; –: No data. MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration.

Table 6
Main phenolic compounds identified by RP-HPLC in ethyl acetate extract of roots from *H. cheirifolia*.

N°	RT (min)	Compounds	% of compounds
1	17.21	*Trans*-cinnamic acid	4.04
2	18.37	Ferulic acid	1.74
3	18.60	Rutin hydrate	3.25
4	18.94	Unknown	3.87
5	19.71	Myricetin	2.51
6	21.04	Unknown	3.21
7	21.53	Unknown	7.79
8	21.58	Naringin	7.75
9	22.03	Unknown	2.85
10	22.10	Unknown	2.38
11	22.46	Unknown	7.93
12	22.53	Naringenin	2.61
13	23.61	Unknown	4.88
14	24.13	Unknown	1.84
15	24.36	Unknown	7.64
16	24.41	Quercetin	4.91
17	25.56	*p*-Coumaric	2.79
18	27.39	Unknown	12.61
19	27.75	Unknown	3.29
20	28.00	Unknown	3.69
21	28.56	Unknown	8.31

RT: retention times (min).

(0.418 ± 0.020) mg/mL. While, the hydro-methanolic, petroleum ether and ethyl acetate extracts were inactive against α-glucosidase.

Basing on the importance of the tested biological activities, chromatographic identification was performed only on the most active extract. The ethyl acetate extract, which was distinguished by the highest antioxidant and antibacterial activities, was selected for the identification of its main compounds by RP-HPLC. Table 6 showed a presence of 21 compounds numbered according to their retention time. In fact, the characterization of this extract qualified eight compounds (Figure 1), namely, *trans*-cinnamic acid (4.04%), ferulic acid (1.74%), rutin hydrate (3.25%), myricetin (2.51%), naringin (7.75%), naringenin (2.61%), quercetin (4.91%) and *p*-coumaric (2.79%). However, the other compounds were unknown. The compound defined by peak 9 (Figure 1) was present in the highest amount in the extract with percentage of 12.61%. Butylated hydroxytoluene, a powerful antioxidant, is added to prevent phenolics degradation during hydrolysis [19]. The majority of known compounds mentioned in Table 6 have been found by several researchers as good antioxidant and antibacterial chemicals [20–26]. Thus, these activities could be attributed to the synergy between the different compounds [27].

4. Discussion

Comparing our results with other studies, Bousselsela *et al.* determined the polyphenol content of the leaves methanolic extract from Algerian *H. Cheirifolia* and detected a content of the order of (30.33 ± 2.82) mg GAE/g [28]. In another study, Benslama *et al.* showed that the polyphenols in the ethyl acetate extract of the leaves was (181.24 ± 14.04) mg GAE/g [29].

The high antiradical property of these extracts may be due to the presence of the phenolic compounds. Indeed, antioxidant

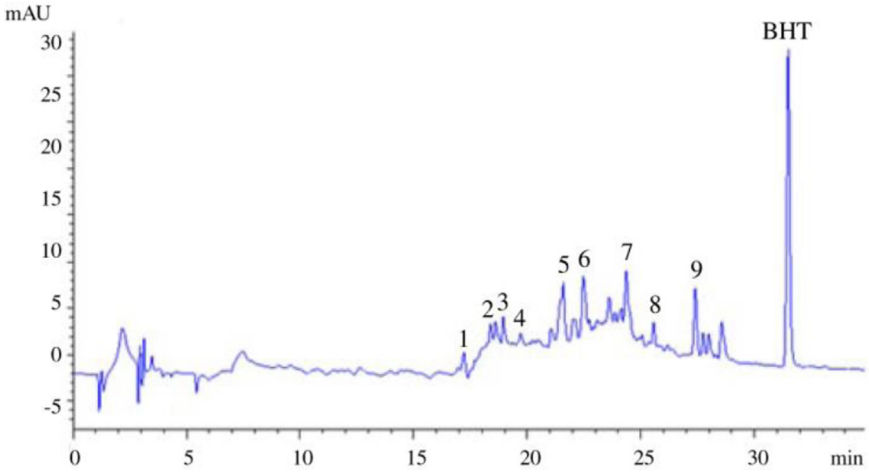

Figure 1. RP-HPLC chromatograms of ethyl acetate extract from *H. cheirifolia* roots.
BHT: Butylated hydroxytoluene.

activities of plants are commonly correlated with their polyphenolic compounds content [30]. In the literature, Benslama *et al.* evaluated the antiradical activity of the *H. cheirifolia* ethyl acetate extract [IC_{50} = (0.149 ± 0.001) mg/mL] [29]. Thus, Bousselsela *et al.* showed that the leaves methanolic extract of this plant has a significant effect against the radical DPPH• and exhibits an important inhibitory activity of β-carotene oxidation [28].

In the literature there are no studies dealing with the antibacterial activity of the *H. cheirifolia* extracts. However, many studies have shown interesting antibacterial activities of the Asteraceae family plants endemic in North Africa. Indeed, Liouane *et al.* reported that the petroleum ether extract of *Cotula coronopifolia* showed significant activity against *Staphylococcus aureus* (MIC = 0.142 mg/mL) and *Escherichia coli* (MIC = 0.250 mg/mL) [31].

The ethyl acetate extract of *Cotula cinerea* exhibited an antibacterial effect with a MIC of 0.2 mg/mL against *Pseudomonas* sp. and *Bacillus* sp. Butanolic extract of this plant was very effective in particular against *Pseudomonas fluorescens* and *Bacillus* sp. with MIC value of 0.012 mg/mL [32,33].

Nevertheless, the α-glucosidase inhibitory activity of flowers extracts from *H. cheirifolia* showed that these extracts have a significant inhibitory effect against this enzyme. Indeed, petroleum ether extract was more potent than the reference product with IC_{50} = (0.242 ± 0.02) mg/mL [8].

Our results revealed that *H. cheirifolia* roots contain different secondary metabolite compounds such as saponins, polyphenols, flavonoids and triterpenes. The ethyl acetate extract displayed antimicrobial property against bacterial strains, as well as significant antioxidant effect especially in DPPH and ABTS assays which could be attributed to the content of phenolic compounds. Butanolic extract showed moderate α-glucosidase inhibitory activity with IC_{50} of (0.418 ± 0.020) mg/mL, while the other extracts were not active.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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