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Polyphenols Contents and Antioxidant Activity of *Hertia cheirifolia* L. Extracts

Mounira Merghem[1*] Meriem Hani[2] and Saliha Dahamna[1]

[1]Laboratory of Phytotherapy Applied to Chronic Diseases,
Department of biology and animal physiology, Faculty of Natur and Life Sciences,
Ferhat Abbas University, Sétif-1, (Algeria)
[2]Laboratory of Natural Resource Valorisation, Faculty of Nature Life Sciences,
Ferhat Abbas University Setif-1, 19000, (Algeria)
Author for Correspondence, mail: mouniramerghem@yahoo.fr

Abstract

Hertia cheirifolia L. is a plant that belongs to the Asteraceae family. It is a medicinal plant traditionally used in the treatment of cramps and stomach pain as well certain gastrointestinal disease. The aim of this study is to evaluate the total polyphenols content and antioxidant activity of the éthanolic extract (EE) and aqueous extract (AqE) of the *Hertia Cheirifolia* L.. Total phenolic and total flavonoid contents was evaluated by colorimetric method and antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching assays. The polyphenols and flavonoids contents of ethanolic extract (EE) is 132.395 µg GAE/mg, 11.95 µg QE/mg and aqueous extract (AqE) is 125.2 µg GAE/mg, 8.25 µg QE/mg, respectively. EE and AqE has scavenging activity for DPPH with IC_{50} 0.241±0.007mg/ml and IC_{50} 0.263 ±0.002mg/ml, respectively. In the β-carotene bleaching test, the AqE exhibited the highest antioxidant activity 71.447 ± 7.549% than EE 67.762 ± 8.987%.

Oxidative stress generated by high free radicals concentrations[19], play a major role in the pathogenesis of several chronic diseases including cancer and neurodegenerative diseases through damaging biological molecules such as proteins, nucleic acids, and lipids[22]. The human body has many pathways to combat oxidative stress by generating antioxidant compounds, either naturally produced in situ or supplied externally by supplements or foods[19]. Antioxidants can inhibit the formation of free radicals in its early stages, or interfere with the propagation reaction of free radical chain reactions. The use of natural antioxidants extracted from plants can reduce the adverse effects of oxidative stress[13].

The Asteraceae is the largest family of flowering plants, comprising more than 1500 genera and 20000 species worldwide. Numerous Asteraceae species are known to have substantial pharmacological proprieties according to their

important phytochemical compounds. *Hertia cheirifolia* L., an endemic Asteraceae species specific to Algerian flora[20], is widely distributed in the North of Africa[4]. This plant has fleshy stems, branched and very leafy 20–40 cm. The leaves are alternate and fleshy. The heterogamous capitula have a big solitary of 2–3 cm in diameter. The flowers are lemon yellow, those peripheral and ligulate are fertile and the others tubular are sterile[12].

Hertia cheirifolia L. is known as medicinal plant and used traditionally to reduce hyperglycemia. Its extracts have demonstrated spasmolytic, acaricidal, antibacterial, antioxidant activities, a-glucosidase inhibition[14]. and anti-inflammatory[15,16].

Chemicals :

Folin-Ciocalteu, aluminum chloride ($AlCl_3$), gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and tween 40 were purchased from Sigma Chemical Co. (St. Louis, MO). Linoleic acid, β-carotene and butylated hydroxytoluene (BHT) were obtained from Fluka Chemical Co. (Buchs, Switzerland).

Plant material :

Hertia cheirifolia L. was collected in March, from Beida Bordj region, Wilaya of Sétif in Northeast of Algeria.

Preparation of plant extract :

Aqueous extract :

The aerial parts of plant material were cleaned with tap water, dried in the shade at room temperature for 2 weeks and ground into powder using an electric grinder. The aqueous extract was prepared by boiling 100g of *Hertia cheirifolia* powder in distilled water for 15 minutes, The resulting mixture was filtered using Wattman filter paper and then evaporated in rotary vacuum evaporator at 45°C.

Ethanolic extract :

The ethanolic extract was obtained by maceration in water/ethanol mixture (20:80) for 24 h. The resultant extract was filtered through Wattman paper and the solvent was removed by rotary evaporator under reduced pressure at 45°C.

Determination of total polyphenol content:

Total phenolic content was determined using Folin-Ciocalteu method, according to Li and al,[11] with slight modifications. A volume of 100 µl of the extract was mixed with 500 µl of Folin–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µl of sodium carbonate solution Na_2CO_3 (75 g/l) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm, Gallic acid (20-140 mg/l) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

Determination of total flavonoids contents:

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method[3]. Briefly, 1 ml of 2% $AlCl_3$ in

methanol was mixed with 1 ml of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) were used as standards for calibration curve and the total flavonoids content was expressed as micrograms quercetin equivalents (QE) per milligram of extract.

Evaluation of antioxidant activity :

DPPH free radical-scavenging assay :

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl(DPPH) assay[6]. After dissolving the aqueous extract in distilled water, the ethanolic extract in ethanol, the solution of DPPH in methanol (0.04mg/ mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration. The mixture was shaken vigorously and then kept in the dark for 30 minutes at room temperature. Then, the absorbance was measured at 517nm. BHT and gallic acid were used as standards. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

Radical scavenging activity (%)=(A_{blank}–A_{sample}/A_{blank}) × 100

A_{blank}: Absorbance of the control.

A_{sample}: Absorbance of the reagent with extract.

β-carotene/linoleic acid assay :

In this test, the antioxidant capacity of the extracts was determined by measuring the inhibition of the oxidative degradation of β-carotene (discoloration or bleaching) by the oxidation products of the acid linoleic[10]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform. One milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added. The chloroform was evaporated using evaporator at 45°C. Then 100 mL of distilled water saturated with oxygen was added. 2.5 mL of this prepared β-carotene solution were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added before incubation for 48h at room temperature. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a positive control and with distilled water and methanol as a negative control. The absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h. The antioxidant activity of extracts was calculated using the following equation:

$AA\% = A_{sample} / A_{BHT} \times 100$.

A_{sample}: Absorbance in the presence of the Extract; A_{BHT} : Absorbance in the presence of positive control BHT.

Statistical Analyses :

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed.

Medicinal plants contain various types of antioxidants, mostly polyphenols and flavonoids which exhibit high antioxidant activity[7]. They have been extensively utilised in traditional practice settings to treat a variety of diseases including cancer, diabetes, hepatitis, tuberculosis and neurodegenerative disorders[21]. Therefor, we have evaluated the polyphenols and flavonoids contents in present study, also the antioxidant activity of *Hertia cheirifolia* L.

extracts. The antioxidant activity was evaluated by the DPPH scavenging activity and β-carotene bleaching tests.

Total polyphenols and flavonoids contents:

Phytochemicals present in plants exhibit health beneficial bioactivities including antioxidant. Phenolic compounds such as tannins, flavonoids and phenolic acids are the major antioxidant phytochemicals[17].

The total phenolic contents were determined using the Folin-Ciocalteu reagent in comparison with standard gallic acid, and the result was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract and the total flavonoids were determined using the aluminum trichloride ($AlCl_3$) method in comparison with standard quercetin, and the result was expressed as micrograms quercetin equivalent (QE) per milligram of extract. The results (Table-1) showed that the total polyphenols and flavonoids content of ethanolic extract (132.395 µg GAE/mg and 11.95 µg QE/mg) were higher than that of AqE (125.2 µg GAE/mg and 8.25 µg QE/mg).

Table-1. Total polyphenols and flavonoids content of *Hertia cheirifolia* L. extracts.

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
AqE	125.2	8.25
EE	132.395	11.95

AqE : aqueous extract, EE : ethanolic extract, GAE: gallic acid equivalent, QE: quercetin equivalent. Each value represents the mean ± SD (n = 3).

Antioxidant activity evaluation :

DPPH radical scavenging activity :

DPPH is a free radical compound which has scavenging ability for antioxidants samples[2]. Is decolorized in the existence of antioxidants and has an unpaired electron which exhibits absorbance of 515 to 517 nm providing a clear dark purple color. Decolorization of DPPH occurs as it accepts electron generated by antioxidant compound, and is quantitatively measured from the changes in absorbance[1].

The concentrations that led to 50% inhibition (IC_{50}) are presented in Table-2. Then, the antioxidant activity were compared with that of BHT and Gallic acid. The AqE and EE showed a scavenging activity which IC_{50} varied from 0.263± 0.002 mg/mL to 0.241±0.007 mg/mL, respectively.

Table-2. DPPH scavenging activity of *Hertia cheirifolia* L. extracts and standards

Extracts	IC_{50}(mg/mL)
AqE	0.263± 0.002
EE	0.241±0.007
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

#: µg/ml. Each value represents the mean ± SD (n = 3).

β-carotene/linoleic acid bleaching assay :

In the β- carotene/linoleic acid system, linoleic acid produces hydroperoxides wich cause rapid discoloration of β-carotene. However, antioxidants from the extracts can be neutralize hydroperoxides formed in this system and protect β-carotene from discoloration

(oxidation)[9]. The antioxydant activity of aqueous extract AqE (71.447 ± 7.549%) was higher than that of the ethanolic extract (67.762 ± 8.987%) (table 3).

Table-3. Antioxidant activity of *Hertia cheirifolia* L. extracts at 24 hours of incubation measured by β-carotene bleaching method.

Extracts	Inhibition %
AqE	71.447 ± 7.549
EE	67.762 ± 8.987
BHT	100 ± 3.972
H_2O	30.91 ± 3.864

Each value represents the mean ± SD (n = 3).

Previous studies recorded that phenolic compounds including flavonoids are associated with strong antioxidant activity and they possess healthy benefits[2]. when a phenolic molecule loses an electron or when it acts as a reducing agent, the molecule becomes a relatively stable radical while its oxidized intermediates can also now become prooxidants[18]. The flavonoids structure, its hydroxyl atom location, and its other properties are important for antioxidant and reactive species neutralizing capacity[8].

Numerous Asteraceae species are known to have substantial pharmacological proprieties according to their important phytochemical compounds such as polyphenols (C glycosyl flavonoids, caffeoylquinic acids, etc.), diterpenes, and sesquiterpenes lactones (eudesmanetype, germacrane-type, drimane-type, *etc*.)[20].

Hertia cheirifolia L. is a species of flowering plants in the Asteraceae family. The leaves of this plant have been used in the traditional medicine to treat rheumatic pains and to reduce hyperglycemia.[16] and exhibited potent antioxidant activity and interesting protective capacity against DNA damage, lipid peroxidation, protein oxidation, and inflammation. The level of biological activity of the plant extract depends mainly on its chemical composition,[20] particularly substitution hydroxy for the aromatic rings A and B and the model of substitution of the ring C, the most active flavonoïds have from 3 to 6 groups of hydroxyls. On the other hand the glycolsylation of the flavonoïds reduces their capacities to trap the free radicals[5].

From the results obtained in this study, we concluded that the extracts of *Hertia cheirifolia* L. had an antioxidant activity in DPPH and β-carotene-linoleic acid bleaching assays.

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