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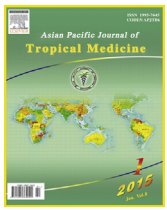

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Phytochemical analysis and biological activities of *Hertia cheirifolia* L. roots extracts

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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 $P < 0.05$.

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