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Research Paper

Effects of water deficit and rehydration on antioxidant and anti-inflammatory activities in methanolic root barks extracts of *Rhus tripartitum* and *Periploca laevigata* subsp. *angustifolia*

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ABSTRACT

Rhus tripartitum (Ucria) and *Periploca laevigata* Aiton subsp. *angustifolia* (Labill.) are two medicinal plants containing several compounds with important pharmacological activities. The obtained results showed that under water deficit, the secondary metabolite levels in root bark were increased significantly for both species and decreased after rehydration for *R. tripartitum* but for *P. laevigata*, the amount of secondary metabolite increased. In addition, the quantification of phenolics and flavonoids compounds by HPLC was found to be in accordance with these results. The main compounds were catechin, cinnamic acid, epicatechin, apigenin and rutin. They also suggest that water deficit and rehydration treatments may regulate the production of phenolic compounds in *Rhus* and *Periploca* root bark. Moreover, the extracts of the root bark from *Rhus* and *Periploca* were able to scavenge DPPH and FRAP free radicals with the low IC50 and EC50 recorded for stressed plants extracts (75.66 µg/ml, 111.66 µg/ml and 118.66 µg/ml, 144.66 µg/ml, respectively for *R. tripartitum and P. laevigata*) and have an inhibitory effect by decreasing NO and ROS release in J774A.1 macrophages. The high antioxidant activity was detected with *R. tripartitum*, while anti-inflammatory activity was important with *P. laevigata*. The increase of these biological activities was directly proportional to the content of secondary metabolite in root bark. In general, water deficit revealed positive effects on secondary metabolites contents, hence the biological potential of *R. tripartitum* and *P. laevigata*.

1. Introduction

In the last years, the interest in the research of natural bioactive compounds from botanical sources has grown. These compounds are used as an alternative of synthetic compounds due to the latter's possible toxicity and carcinogenicity (Hu et al., 2000; Namiki, 1990). Moreover, these natural products are involved in the preservation of human health (Giorgi et al., 2009). Polyphenolic compounds are among the most influential and widely distributed secondary products in the plant (Jwa et al., 2006). Many of them provide the plant with not only the capability to survive under unfavorable conditions, but also the resistance to different stress types (Ayaz et al., 2000).

Water deficit is considered among most important factors that affect growth and lead to metabolic disorders in plants. In general, it has a negative effect on the development of plants. Nevertheless, it has a positive effect as far as the biosynthesis and the accumulation of secondary metabolites. Their biosynthesis in plants is mostly stimulated in response to biotic/abiotic stress (Naczk and Shahidi, 2004). Thus, water deficit can be an approach to increase the production of secondary metabolites in plants and might represent a potential source of polyphenols. Consequently, the optimal polyphenols yield would be obtained using stress-tolerant species (Abreu and Mazzafera, 2005).

Among the known drought-tolerant species we can mention *Rhus tripartitum* (Ucria; Anacardiaceae) and *Periploca laevigata* Aiton subsp. *angustifolia* (Labill.) (Asclepiadaceae), which represent very abundant species in North Africa (Pottier-Alapetite, 1979), especially in the steppes of desert, arid and semi-arid areas. These two species have recently gained increasing attention due to their adaptation to water stress (An et al., 2011) as well as their medicinal value (Chetoui et al., 2013; Zito et al., 2011).

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Rhus tripartitum and *Periploca laevigata* have been widely used as medicinal plants in both modern and traditional medicine. Indeed, the different parts of these herbs have been employed as a remedy for many diseases, such as diarrhea, dysentery (Abbassi and Hani, 2012), rheumatism, mental disorders, diabetes, hemorrhoids, and gastric ulcer (Alimi et al., 2013). The antioxidant (Lee et al., 2002; Hajji et al., 2009), anti-inflammatory, antimicrobial (Hajji et al., 2009; Mahjoub et al., 2010; McCutcheon et al., 1994), antitumor, hypoglycemic (Giancarlo et al., 2006), anti-proliferative (Spera et al., 2007) and hypotensive effects of *Rhus tripartitum* and *Periploca laevigata* have also been investigated.

Up to now and to the best of our knowledge, no studies have been carried out on the effects of water deficit and rehydration on phytochemical, antioxidant and anti-inflammatory activities of root bark from *R. tripartitum* (Ucria) and *P. laevigata* subsp. *angustifolia*. That is why the utmost purpose of the present study is to determine the effect of different water treatments in the level of phenolic compounds and to evaluate the antioxidant and anti-inflammatory activities of *R. tripartitum* and *P. laevigata* subsp. *angustifolia*.

2. Material and methods

2.1. Chemicals

Methanol, ascorbic acid, hydrochloric acid, acetonitrile, acetic acid, and trichloroacetic acid were purchased from RiedeldeHaen (Switzerland). The solvents were of appropriate purity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-tert-butyl-4-hydroxy-boxylic acid (BHT), sodium carbonate (Na_2CO_3), gallic acid, catechin, quercetin, potassium ferricyanide $K_3Fe(CN)_6$, ferric chloride, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), 3-(4,5dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Sigma-Aldrich (Chemie Gmbh, Steinhein, Germany).

2.2. Plant material

Rhus tripartitum (Ucria) and *Periploca laevigata* Aiton subsp. *angustifolia* (Labill.) used during this experiment were two-year-old plants produced by the Bouhedma park (34° 28′ 37″ N 9° 37′ 29″ E). The study was conducted with plants of *R. tripartitum* and *P. laevigata* Aiton subsp. *angustifolia* cultivated from seeds on 5l pots containing peat, compost and sand (3:3:1) in greenhouse conditions (25 °C ± 3, 16/8 h day/night photoperiod, 400–480 µmol $m^{-2}s^{-1}$ light intensity), situated at the Experimental Station of Gafsa Faculty. Experiments lasted 2 months from April 2016 to June 2016.

2.3. Treatments

Sixty pots were separated into two groups for two different water treatments: Control plants were watered to 100% Field capacity (FC) every two days, and stressed plants were subjected to water stress. Water stress was imposed by subjecting plants to a gradual decrease of soil water availability for a maximum period of 30 days, followed by a re-watering period lasting for 7 days.

2.4. Preparation of extracts

The preparation of the extracts was determined according the methods of Tlili et al. (2014). 1 g of fine powder of root bark was extracted with 10 ml of 80% methanol solution for the preparation of methanolic extracts during 24 h. Then, the extract was filtered, evaporated under vacuum to dryness and kept at +4 °C until further analyses.

2.5. Total phenolic contents

Total phenolic content of methanol extracts was determined using the Folin–Ciocalteu reagent according to the method of Dewanto et al. (2002). A volume of 0.5 ml of the diluted methanolic extract solution (8 mg extract/6 ml of 60% methanol solution) was mixed with 2.5 ml of Folin–Ciocalteu's reagent and 2 ml of Na_2CO_3 (7%) solutions. After incubation for 90 min in the dark, the absorbance was measured at 760 nm. For the calibration curve, the diluted solutions of gallic acid were used and the total phenolic contents were expressed in terms of equivalent amounts of gallic acid per gram of dry weight (GAE/g DW).

2.6. High performance liquid chromatography analysis

The analyses were performed in HPLC-Diode-Array Detection (DAD) with a Varian ProStar HPLC System (Varian 330/Vis Detector and Varian 230 SDM). The separation was carried out on a Zorbax column SB-C18 (150 mm × 4.6 mm, particle size 5 µm) reversed phase column. The mobile phase was a 19:1 (v/v) mixture of acetonitrile–water (solvent A) and water-acetic acid (solvent B). The flow rate was 0.5 ml/min and the injection volume was 20 µl at 30 °C. The elution gradient program with a ratio of A to B was as follows: from 0 to 10 min (5:95, v/v), from 10 to 20 min (25:75, v/v), from 20 to 30 min (75:25, v/v), from 30 to 40 min (90:10, v/v) from 30 to 40 min (100:0, v/v). The identification of different compounds detected based on the comparison with the retentions times and by co-injection of authentic standards.

2.7. Total flavonoid contents

The colorimetric assay described by Zou et al. (2004) was used to dose the total flavonoid compounds. Briefly, 1 ml of appropriately diluted methanolic extract was mixed with sodium nitrite ($NaNO_2$, 5%). After 5 min, 1 ml of aluminum chloride ($AlCl_3$, 10%) and 500 µl of sodium hydroxide (NaOH, 1 M) were added to the mixture. The absorbance was read at 510 nm after incubation at room temperature for 15 min. For calibration curve, the dilute solutions of quercetin were used and the total flavonoids content was expressed as mg of quercetin equivalent per gram of dry weight (QE/g DW).

2.8. Condensed tannins contents

The condensed tannins were determined with the vanillin method as developed by Schofield et al. (2001) with a slight modification using a UV–visible spectrophotometer. 1 ml of methanolic extract was mixed with 5 ml of methanol vanillin solution (4%) and 2.5 ml of concentrated hydrochloric acid. After incubation for 20 min at 30 °C, the absorbance of the mixture was measured at 500 nm. Different concentrations of catechin used as calibration curve, and condensed tannins contents were expressed as mg of catechin equivalent per gram of dry weight (CE/g DW).

2.9. Antioxidant activity

2.9.1. DPPH radical scavenging activity

The free radical scavenging assay was determined by 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) (Tlili et al., 2014). Various concentrations of *Rhus tripartitum* and *Periploca laevigata* extracts were prepared (50–250 µg/ml) and 500 µl of each solution was mixed with 3.8 ml of methanol DPPH solution (4×10^{-5} M). The mixture was incubated in the dark at room temperature for 30 min; the absorbance of the solution was measured at 517 nm.

The percentage inhibition (I%) of DPPH free radical scavenging activity calculated from the following formula:

DPPH scavenging effect (%) = $[(A_0\text{-}A_s)/A_0] \times 100$, where A_S is the absorbance of the test sample and A_0 is the absorbance of the DPPH solution.