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Antibacterial activity from *Rhanterium adpressum* flowers extracts, depending on seasonal variations

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ABSTRACT

The main purpose of this study was to evaluate the effect of solvent extraction (acetone or methanol) on total phenolic content and antibacterial potential of *Rhanterium adpressum* flowers extracts. Flowers of the plant were harvested during three different months (April, May and June) for two years 2011 and 2012. The Folin–Ciocalteu method was used to determine the total phenolic content (TPC) in organic extracts; however, the antibacterial activity was tested using disc diffusion method. The highest phenolic content was detected in *R. adpressum* methanolic extracts collected in April. Same was evident in the antibacterial assay and it was also remarkable that the extracts activity was related to the concentration of phenolic compounds in active extracts, antibacterial activity was more efficient against *Staphylococcus aureus* and *Salmonella typhi* Gram + Bacteria. This is the first report on seasonal changes of phenolic compounds and antibacterial activity on *R. adpressum* and might be interesting to prospection of new molecules with antibacterial properties.

Introduction

The genus *Rhanterium* from the *Asteraceae* family is distributed over western North Africa (in Algeria and bordering areas of eastern Morocco), the Arabian Peninsula, Iraq and Iran (Wiklund, 1986). *Rhanterium adpressum*, commonly known in Algeria as "Arfadj", is easily recognized by its broad and is used by the local population in the production of cheese and in folk medicine as an antidiuretic. There are only a few studies in the literature concerning the secondary metabolites of *Rhanterium* species (Bouheroum et al., 2007; Kala et al., 2009; Hamia et al., 2013). Organic extracts (ethyl acetate) from *Rhanterium adpressum* flowers and from the aerial parts of *Rhanterium suaveolens* were shown to be a potential source of antioxidants (Boussoussa et al., 2014; Bouaziz et al., 2009). Similarly to what has been observed for antioxidant properties, antibacterial activity of *R. adpressum* has also been reported. The Essential oils extracted by hydrodistillation from the aerial parts of plant showed antibacterial and antifungal activities against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Fusarium oxysporum* (El-houiti, 2010), whereas phenolic extracts of *R. adpressum* presented a good activity against *Bacillus cereus* and *Enterococcus faecalis* (Boussoussa et al., 2014). In addition, a variety of natural compounds from *Rhanterium adpressum* are identified such as β-Eudesmol, 16 b-Hydroxylupeolyl-3-hexadecanoate and Stigmasterol from chloroform extracts of *R. adpressum* aerial parts (Bouheroum et al., 2007). Also, a high level of Oxygenated monoterpenes and sesquiterpenes such as Spathulenol and β-Eudesmol was determined in *R. adpressum* essential oils (Kala et al., 2009). These results indicated the potential of this plant as a crude drug and dietary health supplement.

Different factors can determine the profile of secondary metabolites in plants. Genotypic characteristics of species and phenology are key elements which determine the phytochemical profile (Fillipini et al., 2010). Biotic (pathogens and herbivory) and abiotic factors (light, temperature, nutrients and water availability), as well as geographical conditions, may directly affect the chemical composition of plant secondary metabolites (Moura et al., 2010; Sousa et al., 2009). Phenolic compounds, including flavonoids, are frequently related to plant-environment interactions, playing a role in UV protection, allelopathy and attraction of pollinators (Grace and Logan, 2000).

To the best of our knowledge, there are no reports demonstrating the antibacterial effects of *Rhanterium adpressum* extracts and the relationship between seasonality and the content on phenolic compounds. Studies on this species are justified by the prospection for new antioxidants and antimicrobial molecules. Thus, the

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Table 1
Microorganisms used in the present study to test antimicrobial activity of *Rhanterium adpressum* extracts.

Strains	Reference	Origin
Escherichia coli	ATCC 25922	LMHD
Pseudomonas aeruginosa	ATCC 27853	LMHD
Staphylococcus aureus	ATCC 29213	LMHD
Salmonella typhi	Isolate	LMHD

objective of this study was to determine the effect of seasonality on the levels of phenolic compounds of *R. adpressum* flowers with the use of two solvent for extraction, and evaluate the antibacterial potential of the plant extracts.

Material and methods

Plant material

R. adpressum Coss. & Durieu was collected in three different months (April, May and June), 2011 and 2012, from the region of Zelfana (located at 660 km SSE of Algiers: latitude 32°23′46”(N); longitude 5°13′34”(E); altitude 354 m) by the Pr YOUSFI Mohamed and it was authenticated by Pr CHAHEMA Abedelmajid (Biology Department, University of Ouargla Algeria). A voucher specimen (RACD47/05/07) was deposited in the herbarium of the Fundamental Sciences Research Laboratory at Laghouat University. The material obtained was dried separately at room temperature and finely grounded into a fine powder.

Extraction

samples of 5 g of dry mass (DM) were extracted with absolute methanol and acetone for (24 h × two times) at room temperature. After filtration, each fraction was evaporated using vacuum evaporator and the extracted fraction was weighed to determine the total phenolic content and then stored at +4 °C for further analysis.

Determination of total phenolics

The amount of total phenolics in methanol and acetone extracts was analyzed in a spectrophotometer, according to the Singleton and Rossi (1965) method . Samples (100 μL, three replicates) were introduced into test tubes containing 500 μL of Folin– Ciocalteu's reagent (1/10 aqueous dilution) and 2 mL of sodium carbonate (2%). The contents in the tubes were mixed and allowed to stand for 30 min. Absorbance at 765 nm was measured in (Shimadzu 1601 UV–vis spectrophotometer). The total phenolic content was expressed as gallic acid equivalents (GAE) per gram of dry weight plant material.

Screening for antimicrobial activity

Seasonal variation of the antibacterial activity of the *R. adpressum* alcoholic extracts was evaluated against two Gram-positive bacteria, *S. aureus* ATCC 25923 and *Salmonella typhi* (isolate), and two Gram-negative bacteria, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (Table 1). The microorganisms were obtained from (Laboratoire de Microbiologie de l'Hopital de Djelfa; LMHD).

Working cultures were prepared by inoculating a loopful of each tested bacteria in 3 mL Muller–Hinton broth and were incubated at 37 °C for 12 h. For the test, the turbidity of the overnight broth was adjusted to 0.5 Mc Farland standards and then diluted in 0.9% NaCl solution to a final inoculum concentration of 10^6 CFU/mL in order to use them in the activity assays.

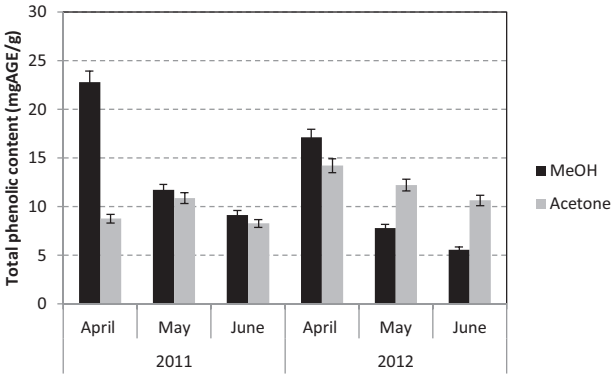

Fig. 1. Effect of various periods of collect with use of two solvent of extraction (methanol and acetone) for two years on the yield of total phenolics in flowers of *Rhanterium adpressum*.

Table 2
Total amount of phenolic compounds in acetonic and methanolic extracts of *Rhanterium adpressum* flowers.

			C (mgGAE/g)
Methanolic extracts	2011	April	22.8 ± 0.53
		May	11.7 ± 0.326
		June	9.15 ± 0.292
	2012	April	17.1 ± 0.464
		May	7.8 ± 0.205
		June	5.55 ± 0.177
Acetonic extracts	2011	April	8.76 ± 0.33
		May	10.87 ± 0.354
		June	10.26 ± 0.362
	2012	April	14.2 ± 0.252
		May	14.2 ± 0.318
		June	12.63 ± 0.349

Antimicrobial tests were carried out according to Cseke et al. (2006). The antimicrobial activity of methanol and acetone crude extracts was determined by the agar disc diffusion method against four microbial strains, as previously described. One hundred microliters of the inocula were spread over plates containing sterile Mueller–Hinton agar (pH 7.2) and paper filter discs (6 mm) impregnated with 10 μL/disc of each extract diluted in dimethylsulfoxide DMSO (10%) were placed on the surface of the media (50, 100 and 150 μg/disc respectively).

The plates were left for 15 min at room temperature to allow the diffusion of the extract and incubated at 37 °C for 18 h. At the end of that period, the inhibition zone around the disc was measured.

Results and discussion

Preliminary experiments were set to assess the effect of the flowering period of plant, plant part and solvent of extraction (acetone or methanol) on productivity of total phenolics and correspondingly antibacterial activity. In all, 12 samples were analysed during the study. Fig. 1, represents the total phenolic content TPC (mg/g) obtained using 2 solvents of extraction and 3 different period of collect (April, May and June) for each sample.

The amount of total phenolics varied in the samples and ranged from 5.55 ± 0,177 to 22.8 ± 0.53 mg/g for methanolic extracts and from 8.76 ± 0.33 to 14.2 ± 0.25 mg/g for acetonic extracts (Table 2). Methanol extract collected in April (2011 and 2012) contained the highest amount of total phenolic compounds. On the other hand, the acetonic extracts possessed low levels of total phenolic content.

The use of two different solvent for extraction certainly affected extraction productivity of phenolic compounds. Though