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Phytochemicals, antioxidant and antifungal activities of *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel.

Lamia Sakka Rouis-Soussi [a], Naima Boughelleb-M'Hamdi [b], Asma El Ayeb-Zakhama [a], Guido Flamini [c], Hichem Ben Jannet [d], Fethia Harzallah-Skhiri [a,*]

[a] Laboratory of Genetic Biodiversity and Valorisation of Bioresources (LR11ES41), High Institute of Biotechnology, Rue Tahar Haddad, Monastir 5000, Tunisia
[b] Department of Biological Sciences and Plant Protection, High Institute of Agronomy of Chott Meriem, University of Sousse, Chott Meriem 4042, Tunisia
[c] Dipartimento di Farmacia, Faculty of Farmaci, Via Bonanno 33, 56126 Pisa, Italy
[d] Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Team: Medicinal Chemistry and Natural Products, Faculty of Sciences, Monastir, Tunisia

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ABSTRACT

The chemical composition of essential oil hydrodistillized from *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel. leaves was analyzed by GC and GC/MS. Nine extracts obtained from flowers, stems and leaves and bulbs and bulblets of *A. roseum* var. *grandiflorum* were tested for their total phenol, total flavonoid and total flavonol content. All these extracts and the essential oils from fresh stems, leaves and flowers were screened for their possible antioxidant and antifungal properties. The results showed that the hexadecanoic acid was detected as the major component of the leaf essential oil (75.9%). The ethyl acetate extract of stems and leaves had the highest antioxidant activity with a 50% inhibition concentration (IC_{50}) of 0.35 ± 0.01 mg/mL of DPPH$^{\bullet}$ and 0.71 ± 0.01 mg/mL of $ABTS^{\bullet+}$. All the extracts appeared to be able to inhibit most of the tested fungi. The essential oil of the leaves had an antifungal growth effect on *Fusarium solani* f. sp. *cucurbitae* and *Botrytis cinerea* (39.13 and 52.50%, respectively). This could be attributed to the presence of hexadecanoic acid, known for its strong antifungal activity. In conclusion, in addition to the health benefits of *A. roseum*, it can be used as an alternative pesticide in the control of plant disease and in the protection of agriculture products.

1. Introduction

Much research has been performed to investigate the phytotherapeutic properties of the *Allium* genus. Recent studies have confirmed the antibacterial, antifungal, antiviral, immuno-stimulating, and antioxidant properties and cholesterol lowering effects of *Allium* species (Pittler and Ernst, 2007; Stajner et al., 2008). Due to their health beneficial effects, extensive scientific investigations have been mainly conducted on the phytochemistry and biological properties of *Allium sativum* L. and *Allium cepa* L. (Corzo-Martinez et al., 2007).

Allium plants and their extracts contain different chemical compounds; an abundance of bioactive constituents namely organo-sulfur compounds, volatile sulfur compounds and proteins. Prostaglandins, fructan, vitamins, polyphenols, fatty acids and essential oils have also been identified (Corzo-Martinez et al., 2007). Because of its secondary metabolite production, in particular because of its sulfur and other numerous phenolic compounds' content, *Allium* species are of great interest (Rivlin, 2001; Griffiths et al., 2002). This genus is also one of the major sources of polyphenol compounds (Shon et al., 2004; Singh et al., 2009). However, polyphenols are bioactive molecules widely distributed in many plant species, with a great variety of structures, ranging from simple compounds to very complex polymeric substances (Pârvu and Pârvu, 2011). Among polyphenols, flavonoids are the best known and best characterized group. This group is further subdivided into classes which include flavones, flavonols, isoflavonoids and proanthocyanidins (Ferguson Lynnette, 2001). The different polyphenol classes share the ability to act as chain-breaking antioxidants, which confers protection against the damage caused by free radicals to DNA, membrane and cell components (Halliwell, 1996; Madhujith and Shahidi, 2009). The total polyphenol content is a good indicator of the antioxidant capacity and various studies have reported a high correlation between antioxidant capacity and this value (Simonetti et al., 1997; Pellegrini et al., 2000). Moreover, polyphenols are also shown to exhibit antibacterial, anti-inflammatory, antiallergenic, antiarthrogenic and antithrombotic effects (Ajila et al., 2010). Different flavonoids have showed distinct antioxidant and antibacterial activities (Akroum et al., 2010).

Although antioxidant activity of some *Allium* species has already been reported elsewhere (Yin and Cheng, 1998), little attention was paid to their antifungal potential (Yin and Tsao, 1999). Garlic extracts

* Corresponding author at: High Institute of Biotechnology of Monastir, University of Monastir, Rue Tahar Haddad, 5000 Monastir, Tunisia. Tel.: +216 73405405; fax: +216 73405404.
E-mail address: fethiaprosopis@yahoo.fr (F. Harzallah-Skhiri).

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have been demonstrated to have antifungal properties against soil borne fungal pathogens (Sealy et al., 2007).

Allium roseum L. is a bulbous perennial plant native from the Mediterranean. *A. roseum* var. *grandiflorum* Briq. has big flowers with large obtuse tepals. Leaves are flattened, 5–10 mm wide, papillose on the edges; it grows on undergrowth and grassy slopes. The subvariety *typicum* Regel. is characterized by inflorescences that do not have bulblets with well developed flowers. In Tunisia, this subvariety is widespread in the North East and the North West of the country and on mountainous regions located in the Center and has also reached the South (Cuénod et al., 1954). Le Floc'h (1983) reported that *A. roseum* was used since ancient times as a vegetable, spice or herbal remedy to treat headache and rheumatism.

Investigations dealing with *A. roseum* growing wild in Tunisia are scarce. Recently, some studies have described the chemical composition of the essential oil and the antimicrobial and antioxidant activities of *A. roseum* var. *odoratissimum* (Desf.) Coss. collected in certain regions of Tunisia (Najjaa et al., 2007, 2011; Dziri et al., 2012; Zouari et al., 2012, 2013). Nevertheless, no information concerning the *A. roseum* var. *grandiflorum* growing wild in Tunisia has been published, except the work of Ben Jannet et al. (2007) who studied the chemical composition of essential oils of flowers and stems of the same species as reported upon in this study.

Therefore, the aim of the present work was to study the phytochemical content of the essential oil and organic extracts of *A. roseum* var. *grandiflorum* subvar. *typicum* Regel. and their antioxidant and antifungal properties used in the Tunisian traditional medicine or generally as food.

2. Materials and methods

2.1. Plant material

A. roseum var. *grandiflorum* subvar. *typicum* was collected in the region of Sousse (coastal region, in the Center-East of Tunisia, geographical coordinates are 35°49′32″ North, 10°38′28″ East), during their blooming stage in March 2007 (for the extraction of organic extract) and in March 2011 (for the extraction of essentials oils). Identification was performed according to the "Flora of Tunisia" (Cuénod et al., 1954), by the botanist Pr. Fethia Harzallah-Skhiri and a voucher specimen (Al.104) has been deposited in the laboratory of Genetic Biodiversity and Valorisation of Bioresources, High Institute of Biotechnology of Monastir, Tunisia.

2.2. Preparation of A. roseum var. grandiflorum organic extracts

Plant samples were separated in three parts: (flowers), (both stems and leaves) and (both bulbs and bulblets). Fresh flowers and both stems and leaves of *A. roseum* var. *grandiflorum* were air-dried for five weeks then ground into fine powder, whereas the bulbs and bulblets were used fresh. One hundred grams of each plant part was extracted separately at room temperature with 200 mL of acetone–H_2O mixture (8:2 v:v). Extraction was performed twice for 5 days at room temperature. The resulting extract was filtered and the solution was evaporated to remove acetone under reduced pressure in a rotary evaporator (Büchi Rotavapor R-200, Büchi Heating Bath B-490). The remaining aqueous solution was extracted sequentially with the following solvents: chloroform, ethyl acetate and butanol. The extracts were separately concentrated with a rotary evaporator under reduced pressure and stored at 4 °C until tested.

2.3. Preparation of A. roseum essential oils

The fresh stems, leaves and flowers (100 g of each sample in 300 mL of distilled water) were separately submitted to hydrodistillation in a Clevenger-type apparatus for 4 h. The essential oils were collected, dried over sodium sulfate, weighed and stored in sealed glass vials in a refrigerator at 4 °C until use.

2.4. Phytochemical analysis

2.4.1. Analytical GC

Gas chromatograph: HP 5890-series II instrument equipped with a flame ionization detector (FID), HP-5 (30 m × 0.25 mm ID, 0.25 μm film thickness) fused silica capillary column, carrier gas nitrogen (1.2 mL/min). The temperature oven was programmed from 50 °C (1 min) to 280 °C at 5 °C/min (1 min). Injector and detector temperatures were 250 °C and 280 °C, respectively. Volume injected: 0.1 μL of 1% hexane solution. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRIs) relative to the series of *n*-hydrocarbons.

2.4.2. Analytical GC–MS

GC–EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data. Moreover, the molecular weights of all the identified substances were confirmed by GC–CIMS, using MeOH as CI ionizing.

2.4.3. Total phenolic content

The content of total phenolic compounds in organic extracts was measured with a spectrophotometry method based on a colorimetric oxidation/reduction reaction. The oxidizing agent was the Folin–Ciocalteu's phenol reagent (Merck) (Singleton et al., 1965; AOAC, 1984). 50 μL of each diluted organic extract was added to 750 μL of distilled water/Folin–Ciocalteu solution (28:2 v:v). After 3 min, 200 μL of sodium carbonate solution (20%) was added. The reaction mixture was kept in a boiling water bath for 1 min. For the control, 50 μL of methanol was used. The absorbance was measured at 765 nm. Tests were carried out in triplicate. Quantification was obtained by reporting the absorbance in the calibration curve prepared with gallic acid, results are expressed as mg of gallic acid equivalents (GAE) per 100 g Dry Weight (DW).

2.4.4. Total flavonoid content

Total flavonoid content in each organic extract was determined using a spectrophotometric method (Lamaison and Carnat, 1991) with slight modifications, based on the formation of the complex flavonoid–aluminum. To 0.5 mL diluted organic extract, 0.5 mL of 2% aluminum chloride ($AlCl_3$) dissolved in methanol was added. The sample was incubated for 15 min at room temperature and the absorbance of the reaction mixtures was measured at 430 nm. Rutin was used as standard flavonoid. The total flavonoid content was expressed as mg of rutin equivalents per 100 g DW, by using a standard graph and the values were presented as means of triplicate analyses.

2.4.5. Total flavonol content

Total flavonol content in each organic extract was determined according to Miliauskas et al. (2004) with slight modifications. One milligram of each the organic extract was dissolved in 1 mL of methanol. One milliliter of this solution was added to 1 mL of 2% $AlCl_3$ dissolved