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Phytochemical and Antioxidant Study of the Hexanoic Extract of *Rhaponticum acaule* †

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Abstract: The purpose of this work was to study the phytochemical screening and the antioxidant activity of the hexanoic extract of the root part of *Rhaponticum acaule*. The qualitative phytochemical analysis of the extract showed that it is composed mainly by terpenoids. The result of the evaluation of the antioxidant activity revealed that the hexanoic extract is endowed with a very powerful antioxidant activity characterized by inhibitory concentrations of 50% of the free radical DPPH (IC_{50} = 12.5 μg/mL) better than the ascorbic acid (IC_{50} = 23.5 μg/mL). In fact, these new results could lead to powerful approaches for the development of new antioxidant compounds.

Keywords: *Rhaponticum acaule*; phytochemistry; antioxidant

1. Introduction

Antioxidants are substances that delay or inhibit the oxidation of a substrate when they are present in low concentrations [1]. The body possesses a wide range of endogenous antioxidants, either in the form of enzymatic systems or non-enzymatic systems, such as thiols, glutathione, bilirubin, urate and various nutritional components, including certain vitamins. A free radical is defined as a chemical species possessing an unpaired electron in its outer orbit [2]. It is this unpaired electron that makes the free radical unstable and, therefore, quite reactive because it tends to react with other molecules in order to pair this electron and create a more stable species. The most important free radicals in biologic systems are derivatives of oxygen. Free radicals can cause many adverse reactions in vivo that result in cell injury or dysfunction and subsequent inflammation and degenerative disease states [3].

Plants are a source of both structural and functional foodstuffs for humans and animals. They also contain numerous active ingredients employed for medicinal purpose. The Asteraceae family is a large family of dicotyledonous plants comprising almost 20,000 species in 1100 genera, and about 13 tribes [4]. In Algeria, there are 109 genera and 408 species. This vast family is economically important, providing food and pharmaceutical plants. One of the typical properties of the Asteraceae family is its richness in various natural compounds (terpenoids, flavonoids, alkaloids, etc.) [5]. *Rhaponticum acaule,* belonging to the Asteraceae family, is a North African plant common throughout northern Algeria, particularly in sandy coastal locations. Local names include: tafgha, tafraït [6]. The plant is known for its therapeutic properties and is recognized for its positive effects on liver cells, it is an aperitif, cholagogue, stomatal, tonic, etc. Gas chromatography-mass spectroscopy of the essential oil obtained from *Rhaponticum acaule* roots shows the presence of aliphatic alcohols, which constitute the high class (69.2%), followed by terpenes (5.5%), alkenes (5.2%) and alkynes (4.0%) [7].

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2. Material and Methods

2.1. Extraction of Rhaponticum acaule Roots

Air-dried roots of *Rhaponticum acaule* were harvested during March 2020 from the Bensakrane station (Algeria). Extraction by maceration of the roots was carried out under agitation at room temperature, using hexane as the extraction solvent.

2.2. Phytochemical Tests

The following methods were used to carry out a qualitative phytochemical screening.

- Alkaloids: The tests are performed by precipitation reactions with Mayer and Wagner reagents. 1 mL of the hexanoic extract is divided into two equal volumes, one treated with 0.5 mL of Mayer's reagent, the other with 0.5 mL of Wagner's reagent. The appearance of a white or brown precipitate, respectively, reveals the presence of alkaloids.
- Tannins: The presence of tannins is determined by adding 1 mL of water and 1 to 2 drops of 1% $FeCl_3$ solution to 1 mL of the extract. The appearance of a dark green or blue-green color indicates the presence of tannins. The appearance of a dark green color indicates the presence of catechic tannins. Blue-green coloration indicates the presence of gallic tannins.
- Flavonoids: Place 1 mL of the extract in a test tube; add 1 mL hydrochloric acid (HCl) and 3 magnesium chips. The appearance of a red or yellow coloration reveals the presence of flavonoids.
- Saponins: Place 10 mL of the hexanoic extract in a test tube and shake for a few seconds, then leave to stand for 15 min. A persistent foam height indicates the presence of saponins.
- Terpenoids: 5 mL of extract is added to 2 mL of chloroform and 3 mL of concentrated sulfuric acid (H_2SO_4). The formation of two phases and a brown color at interphase indicate the presence of terpenoids.

2.3. Antioxidant Activity

The antioxidant activity was performed through two methods: the DPPH free radical scavenging assay and the Ferric reducing antioxidant power assay, using ascorbic acid as a positive control.

2.3.1. DPPH Free Radical Scavenging Assay

DPPH is a relatively stable free radical that absorbs in the UV-Visible range at 515–520 nm. In order to measure or verify the free radical scavenging capacity of molecules known as antioxidants, DPPH is used, which turns yellow when reduced. According to the protocol described by Que et al. [8], 1 mL of different sample concentrations was mixed with 1 mL of DPPH ethanolic solution (0.1 mM). After 30 min of incubation at room temperature and in the dark, antioxidant activity was measured at 517 nm against blank and standard antioxidant Ascorbic acid. The percentage of anti-free radical activity was calculated according to the following equation:

$$(\%) = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

where: Abs: absorbance

IC_{50} represents the sample concentration required to reduce 50% of the DPPH free radical, determined graphically by linear regression of the graph plotted (inhibition percentages as a function of different sample concentrations). All samples were done in triplicates.

2.3.2. Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP method is based on the reduction of ferric iron Fe^{3+} (yellow color) to ferrous iron Fe^{2+} (blue-green color) in the presence of antioxidants. The intensity of the coloration is measured spectrometrically at 700 nm. Following the protocol of Oyaizu [9], 0.5 mL of the samples at different concentrations are mixed with 1.25 mL of a buffer solution of pH = 6.6 and 1.25 mL of a potassium ferrocyanide solution (1%). The mixture is incubated for 20 min at 50 °C. After cooling to room temperature, 1.25 mL of trichloroacetic acid solution (10%) is added and centrifuged for 10 min. 0.5 mL of the supernatant is added to 1.25 mL of distilled water and 0.25 mL of $FeCl_3$ solution (0.1%). Absorbance readings of the various samples are taken using a spectrophotometer at 700 nm. Analyses were achieved in triplicates.

3. Results and Discussions

The hexanoic extract of the root part of *Rhaponticum acaule* afforded a light yellow color extract with a yield of 5% (2 g) based on the dry mass of the plant.

3.1. Phytochemical Study

To the best of our knowledge, the results obtained in this study are the first published data concerning the phytochemical study and the antioxidant activity of the hexanoic extract of the roots of Rhaponticum acaule. According to Table 1, the qualitative phytochemical analysis of this plant indicates a predominant presence of terpenoids. Additionally, we observed the presence of alkaloids in the hexanoic extract. While the final results revealed the absence of tanins, flavonoids and saponins.

Table 1. The results of the phytochemical screening.

Alkaloids	Tanins	Terpenoids	Flavonoids	Saponins
+	−	++++	−	−

++++: Strongly positive; +++: Moderately positive; +: Weakly positive; −: Negative.

3.2. Antioxidant Activity

3.2.1. DPPH Free Radical Scavenging Assay

The results are presented in Figure 1. A comparison of the DPPH scavenging activity of the tested hexanoic extract with that exhibited by the positive control, ascorbic acid, revealed that the extract displayed significantly stronger activity (IC_{50} = 12.5 µg/mL) than the reference Ascorbic acid (IC_{50} = 23.5 µg/mL), making it approximately twice as effective.

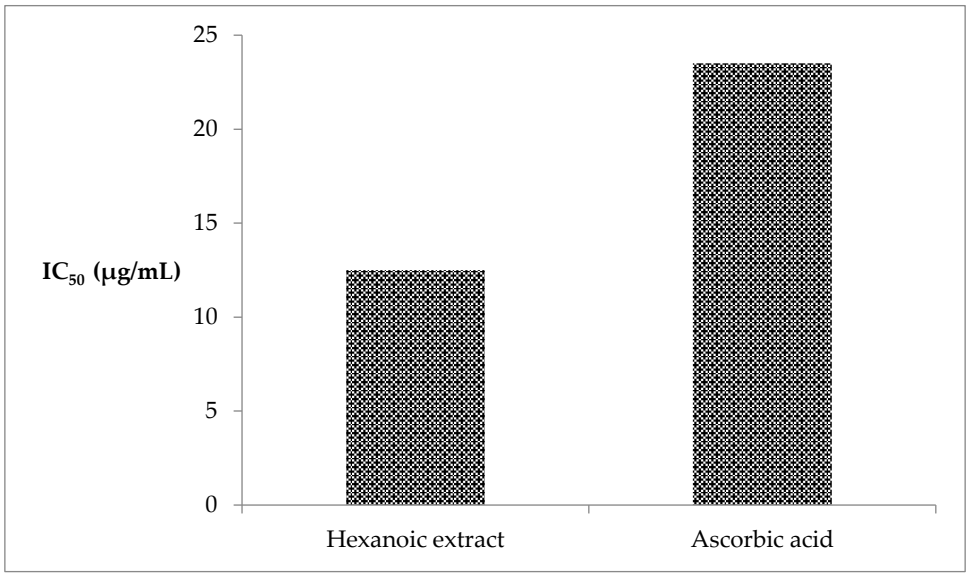

Figure 1. IC_{50} of the hexanoic extract and Ascorbic acid by the DPPH test.

3.2.2. Ferric Reducing Antioxidant Power Assay (FRAP)

Figure 2 represents the absorbance values as a function of the different concentrations of the hexanoic extract and the reference ascorbic acid. The antioxidant activity was found to increase with increasing concentrations. Notably, at higher concentrations, the extract exhibited an antioxidant capacity similar to that of ascorbic acid.

In the present study, the hexanoic extract of *Rhaponticum acaule* showed strong antioxidant activity compared to the synthetic antioxidant, Ascorbic acid. These promising results should be investigated to develop some new antioxidant agents derived from natural sources.

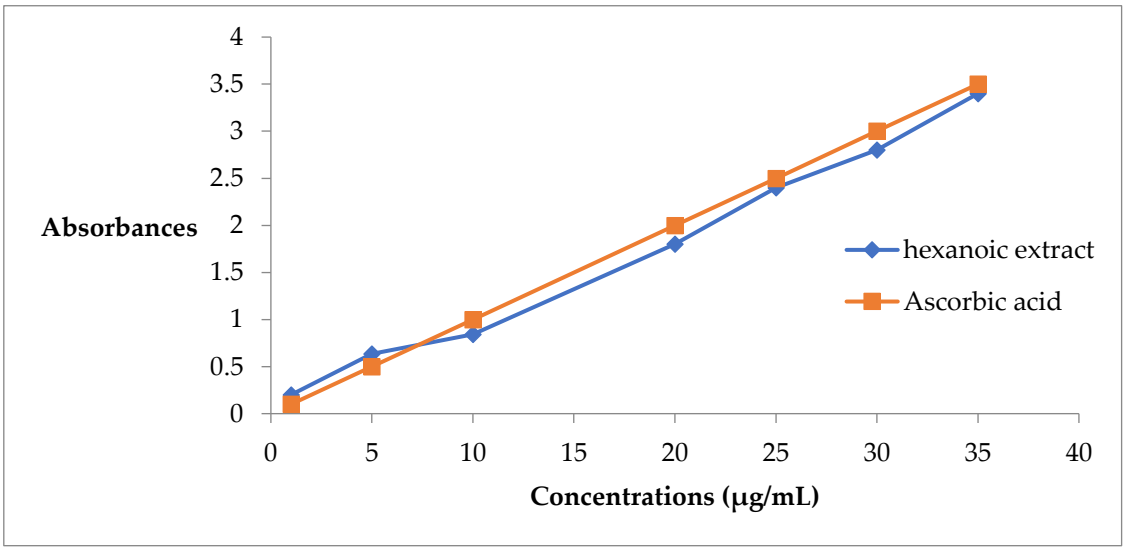

Figure 2. Reducing power activities of the hexanoic extract and Ascorbic acid.

4. Conclusions

This is the first report on the phytochemical study and the antioxidant activity of the hexanoic extract of the roots of *Rhaponticum acaule*. The phytochemical screening has shown that the hexanoic extract was rich in terpenoids. The in-vitro antioxidant activity of the extract revealed better reducing power of the free radical DPPH compared to the reference antioxidant, Ascorbic acid. Therefore, further research is warranted to investigate the chemical composition of the hexanoic extract and perform additional assessments of its therapeutic efficacy.

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