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Research Article

In-vitro Antioxidant and anti-inflammatory activities valorisation of tannin crude extract of *Helianthemum helianthemoides* (Desf.) Grosser

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ABSTRACT

Plants have always been used by humans to relieve and cure many diseases unfortunately the majority of them still unknown. The aims of this study were to evaluate the antioxidant and anti-inflammatory properties of tannin crude extract of *Helianthemum helianthemoides* (Desf.) Grosser. The antioxidant activity was performed by DPPH radical scavenging method and the reducing power assay; however, the anti-inflammatory activity was tested with the Human Red Blood Cell (HRBC) membrane stabilization method. The obtained results indicated high antioxidant potential and a perfect anti-inflammatory agent.

Keywords: *Helianthemum helianthemoides*; DPPH; reducing power; HRBC

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INTRODUCTION

Helianthemum is a genus of plants including around 110 species of evergreen and semievergreen shrubs and belongs to the Cistaceae family also known as rock rose, the sun rose and rush rose. This genus can be found in America, Europe, and Northern Africa. However, the Mediterranean region is considered it's center of diversity [1].

Helianthemum helianthemoides (Desf.) Grosser., or *Helianthemum Fontanesii* B. et R., *H. vulgare* Pers. var. *Fontanesii* B. et T. is characterized by elliptical leaves, acute and revolted, up to 2.5 cm long. The upper face is very green and having, only, very long white hairs spread and a little scattered. The underface white tomentose felting very thick stellate hairs, with marked median nervure, ciliate and hispid. Pedicels and chalice bristling with long white hairs; those of the calyx located on the nervure and going from nervure to nervure or even longer. Intercostal parts of the calyx with thick foliage of yellowish starry hairs. Inflorescence shortly pedunculated, densiflora. Pedicels equaling approximately the chalice. Spatulate epicalice pieces are linear, very hirsute and twice as short as the sepals. Sepals oval and mucronate measured approximately 1 cm. Petals yellow or white of about 1.2 cm. Tomentose capsule, ovoid and much shorter than the calyx. Seeds

compressed, finely granulated. This species grows on limestone rocks of low and medium mountains [2].

Some of the *Helianthemum* species are important medicinal plants used in several countries for different purposes [3 – 7]. This genus is reported to possess anti-inflammatory, antimicrobial, antiprotozoal and antioxidant properties [8 – 12]. Even if this genus was not studied much from the phytochemical viewpoint, some species have been previously examined for bioactive components like flavonoids, phenolic acids, lignans and essential oils [13 – 18].

Therefore, the goal of the present work was to determine the *In-vitro* Antioxidant and anti-inflammatory activities of this endemic species to enrich the Algerian pharmacopeia.

MATERIAL AND METHODS

Plant material

The random sampling was used during the harvesting. The areal parts of *Helianthemum helianthemoides* (Desf.) Grosser., were taken from the mountain of Megriss (X: 5° 18' 20" and X': 5° 24' 7", Y: 36° 18' 30" and Y': 36° 21' 54"). Determined in Laboratory of National Institute of Agricultural Research – Setif – Algeria.

Tannins extraction

Powdered materials (10 g) were macerated in 100 mL of acetone 70 % for 24 hours; the supernatant was then separated from the residue by filtration using Whatman no.1 filter paper and defatted by petroleum ether three times. The resultant fraction was concentrated and dried to a constant weight in a vacuum oven at 45°C and the residues obtained were stored in a refrigerator.

Determination of Total Phenolic Content

For total polyphenol determination, the Folin-Ciocalteu method was used [19]. The samples (0.2 mL) were mixed with 1 mL of the Folin-Ciocalteu reagent previously diluted with 10 mL of deionized water. The solutions were allowed to stand for 4 minutes at 25 °C before 0.2 mL of a saturated sodium carbonate solution (75 mg/mL) was added. The mixed solutions were allowed to stand for another 120 minutes before the absorbance at 765 nm was measured. Gallic acid was used as a standard for the calibration curve. The total phenolic compounds content was expressed as mg equivalent of Gallic acid per gram of extract (mg EAG/GE).

DPPH Assay

The donation capacity of extract was measured by bleaching of the purple colour solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al.*, (1998) [20]. One millilitre of the extract at different concentrations was added to 0.5 mL of a DPPH-methanol solution. The mixtures was shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solutions were then measured at 517 nm. The antiradical activity was expressed as IC₅₀ (micrograms per millilitre). The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH \text{ scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where:

A₀: the absorbance of the control at 30 minutes.

A₁: is the absorbance of the sample at 30 minutes. BHT was used as standard [21].

Reducing power

The reducing power was determined according to the method of Oyaizu (1986) [22]. The extract at different concentrations (2.5 mL) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide. The mixtures were incubated at 50 °C for 20 minutes; after cooling, 2.5 mL of 100 mg/mL trichloroacetic acid were added. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of ferric chloride (1 mg/mL). The absorbance was measured at

700 nm against a blank. A higher absorbance indicates a higher reducing power. EC₅₀ value (mg extract/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from the linear regression analysis. BHA was used as standard [23].

The Human Red Blood Cell (HRBC) membrane stabilization method

The HRBC suspension was prepared. The principle involved here was the stabilization of the human red blood cell membrane by hypo tonicity induced membrane lysis. The mixture contain 1 mL phosphate buffer (pH 7.4, 0.15 M), 2 mL hypo saline (0.36 %), 0.5 mL HRBC suspension (10 % v/v) and 0.5 mL of the plant extract or the standard drug (diclofenac sodium) at various concentrations (10, 50, 100, 250, 500 µg/mL). The control was distilled water instead of hypo saline to produce 100 % haemolysis.

The mixtures were incubated at 37 °C for 30 minutes and centrifuged at 2500 rpm for 5 minutes. The absorbance of haemoglobin content in the suspensions was estimated at 560 nm. The percentage of haemolysis of HRBC membrane can be calculated as follows:

$$Hemolysis (\%) = (Optical \text{ density of Test sample} / Optical \text{ density of Control}) \times 100$$

However, the percentage of HRBC membrane stabilization can be calculated as follows:

$$Protection (\%) = 100 - [(Optical \text{ density of Test sample} / Optical \text{ density of Control}) \times 100] [24].$$

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analysed using t test of Student as primary test followed by Fisher test with the criterion of P < 0.05 to determine whether there were any significant differences between tannins crude extract of *Helianthemum helianthemoides* (Desf.) Grosser., and standards, using Graphpad prism 5 Demo Software.

RESULTS AND DISCUSSION

The results revealed that phenolic content was 69,94±2,77 mg EAG/GE. Polyphenols help in protecting cells against oxidative damage caused by free radicals due to their redox properties, which enable them to act as reducing agents, hydrogen donors and singlet oxygen quencher [17].

The hydrogen atoms or electron donation ability of the extract was measured from the bleaching of purple colour of methanol solution of DPPH [25]. Figure 1 shows the dose-response curve of DPPH radical scavenging activity of tannin crude extract of *Helianthemum helianthemoides*, compared with BHT.

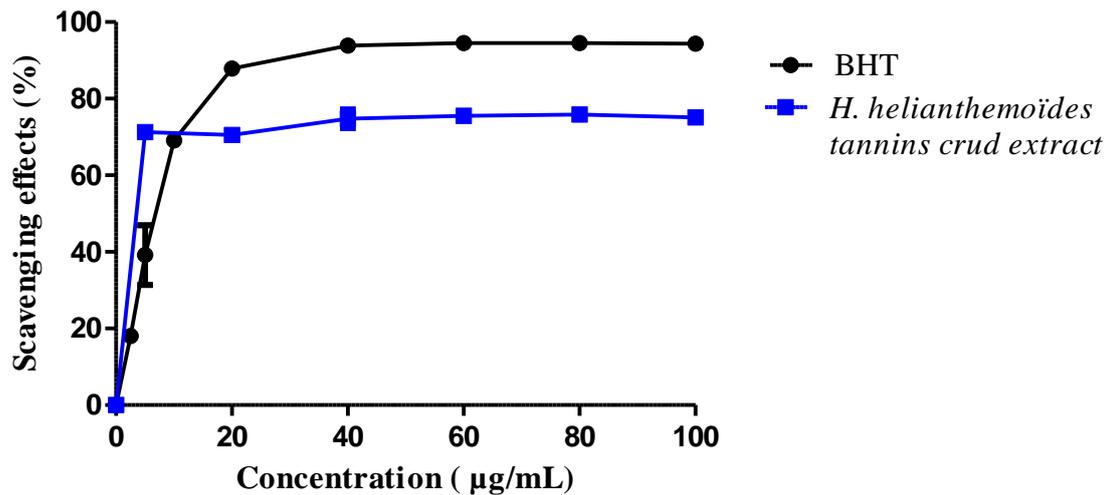


Figure 1: DPPH test of tannins crude extract of *Helianthemum helianthemoides* (Desf.) Grosser

The percentage of scavenging activity and IC₅₀ value of extract were found to be $73,51 \pm 0,95\%$ and $1,56 \pm 0,55 \mu\text{g/mL}^{***}$ respectively against $99,50 \pm 0,01\%$ and $8,76 \pm 0,69 \mu\text{g/mL}$ for BHT. These findings suggested that there could be a correlation between the antioxidant activity and phenolic compounds; and Several authors [26, 27] have well and widely studied their antioxidant properties.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [28]. Figure 2 shows the dose response curves for the reducing powers of tannins crude extract of *Helianthemum helianthemoides*:

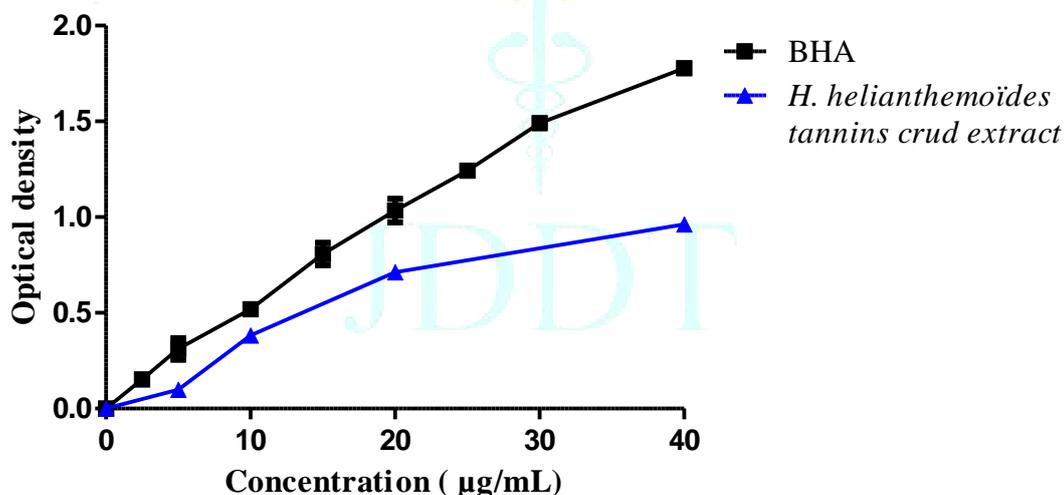


Figure 2: Reducing power activity of tannins crude extract of *Helianthemum helianthemoides* (Desf.) Grosser

It was found that the reducing power increased with the concentration of the sample estimated important, but it still weaker than the standard with EC₅₀ of $17,53 \pm 0,57 \mu\text{g/mL}^{***}$ against $9,03 \pm 0,62 \mu\text{g/mL}$ for the ascorbic acid.

The human erythrocyte membrane lysis induced by a hypotonic solution was used to measure the anti-inflammatory potential of tannins crude extract of *Helianthemum helianthemoides*. Figure 2 demonstrated the ability of the extract to inhibit lysis induced by water.

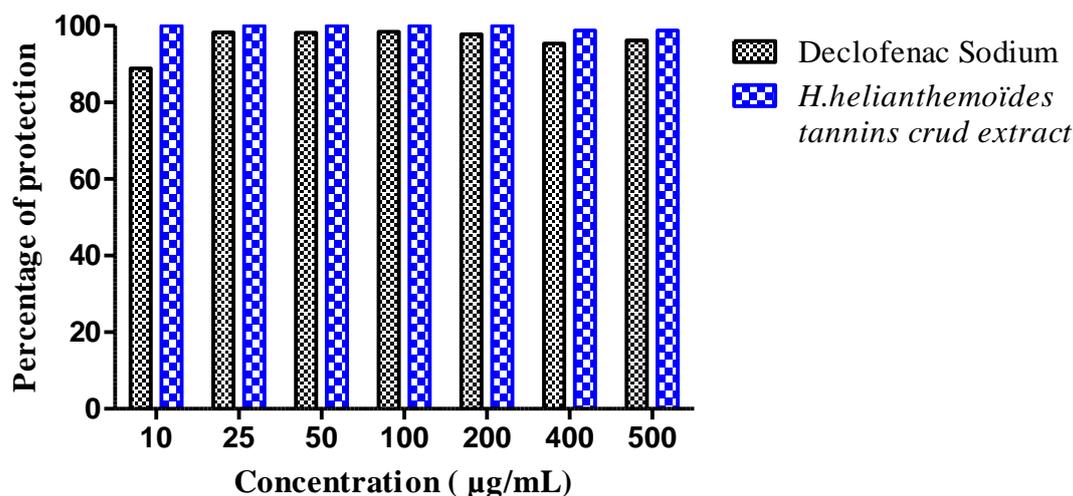


Figure 3: The percentage inhibition of hypotonicity induced hemolysis of HRBCs (%) of standard and tannins crude extract of *Helianthemum helianthemoïdes* (Desf.) Grosser.

The erythrocyte membrane resembles lysosomal membrane and as such, the erythrocyte could be extrapolated to the stabilization of the lysosomal membrane [29]. The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium [30]. Tannins crude extract of *Helianthemum helianthemoïdes* shows a high percentage of protection against hemolysis reach 100%. This is in line with the report of Ahmadiani et al., [31] who stated that flavonoids as well as tannins possess anti-inflammatory effects.

CONCLUSION

To the best of our knowledge, *Helianthemum helianthemoïdes* was studied for the first time. It was found that tannins crude extract of this species possesses a potent antioxidant and anti-inflammatory activities estimated to be perfect. The tannins crude extract of *Helianthemum helianthemoïdes* has shown the presence of an important quantity of polyphenols that may be responsible for the antioxidant and RBC membrane stabilization activities. Additional studies are needed to characterize the active compounds and to clarify the in vivo potential of this plant.

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