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Research Article

IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES VALORISATION OF TANNIN CRUDE EXTRACT OF *GERANIUM ATLANTICUM* B. et R

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ABSTRACT

Tannins are known to be an important group of polyphenol to treat different health problems. The genus Geranium is known to contain flavonoids, tannins, anthocyanidins, lignans, sterols, and polyphenolic compounds. Hence, this study aimed to determine the antioxidant (DPPH) and anti-inflammatory activities **(**HRBC**)** of crude extract of tannin of *Geranium atlanticum*. The results showed an important source of polyphenols, an excellent antioxidant agent and a perfect anti-inflammatory substance.

Keywords: Geranium, Tannins, DPPH**,** Anti inflammatory, Antioxidant.

INTRODUCTION

The genus Geranium L. (Geraniaceae) comprises about 400 species distributed in temperate areas and tropical mountains throughout most of the world. Perennial ground cover plants as floral resources for urban pollinators: A case of *Geranium* species (Masierowska *et al*., 2018). According to Quezel *et al*. (1962), *Geranium atlanticum* B. et R., or *"lbraterraaï "is* a North African endemic species characterised by a red purple intense flower, rarely white. A cylindrical knotty rhizome a tall stem with leaves ramified in general. Theirs speared whitish dense porosity of pedicels. The bracts inflorescence measure 3-5 mm with reddish membranes this species can be found in mountainous areas of Algeria (Quezel *et al*., 1962). The genus Geranium is known to contain flavonoids, tannins, anthocyanidins, lignans, sterols, and polyphenolic compounds and essential oils (Girard *et al*., 2019; Graca *et al*, 2016; Ivancheva & Petrova, 2000; Li *et al*., 2008; Renda *et al*., 2016; Sohretoglu *et al*., 2012; Sohretoglu *et al*., 2009, 2011; Tuominen *et al*., 2015). Tannins are plant secondary metabolites which are known to strongly complex with proteins (Shang *et al*., 2019). They are polyphenolic compounds that are usually categorized into hydrolyzable tannins (HT) and condensed tannins (CT). Condensed tannins, also known as proanthocyanidins are composed of polymers of 2-50 (or more) flavonoid units joined by carbon-carbon bonds. Tannins have many biological activities, such as anticancer, anti-oxidant, anti-inflammatory, anti-asthmatic, and antimicrobial activities (Li *et al*., 2007). The present study focuses on *G. atlanticum* to determine the antioxidant and anti-inflammatory activities of crude extract of tannin, consequently, find a new natural substance which may replace the synthetic ones.

MATERIALS AND METHODS

Plant material

The random sampling was used during the harvesting. The areal parts of *G. atlanticum* 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 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

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resultant fraction was concentrated and dried to a constant weight in a vacuum oven at 45°C and the residues obtained was stored in a refrigerator.

Determination of Total Phenolic Content

For total polyphenol determination, the Folin-Ciocalteu method was used (Hatano *et al*.,1988). 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 were 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-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of (Higuchi, Ishii, & Hanato, 1998). One millilitre of the extract at different concentrations was added to 0.5 mL of a DPPH-methanol solution. The mixtures were shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solutions was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per millilitre). The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_0 – A_1)/ A_0] \times 100$

Where:

A_0: the absorbance of the control at 30 minutes.

A_1: is the absorbance of the sample at 30 minutes. BHT was used as standard (Oyaizu, 1986).

Reducing power

The reducing power was determined according to the method of (Oyaizu, 1986). The extract at different concentration (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 mixture was 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. EC_{50} value (mg extract/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. BHA was used as standard (Chippada *et al*., 2011).

The Human Red Blood Cell (HRBC) membrane stabilization method

To prepare the HRBC suspension, fresh completely human blood (10 mL) was collected and transferred into the centrifuge tubes. These lasts were centrifuged at 3000 rpm for 10 minutes thrice and washed with an equal volume of normal saline each time. The volume of blood was measured and reconstituted as 10 % v/v suspension with normal saline. The principle involved here was stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The mixture contains 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 plant extract or 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:

Haemolysis (%) = (Optical density of Test sample / Optical density of Control) ×100

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

Protection (%) = 100 – [(Optical density of Test sample / Optical density of Control) × 100] (Seema *et al*., 2011)

Statistical analysis

Results were expressed as the mean ± standard deviation. Data were 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 methanol extract of *G. atlanticum*, and standards, using Graphpad prism 5 Demo Software.

RESULTS AND DISCUSSION

The used extraction method provides a yield of crude tannin extract of 16.83 % contains 64,48±3,55 mg EAG/GE, lower than the results found in methanol extract of this species (82,23 ±1,19 mg EAG/GE). The antioxidant properties of tannin crude extract obtained from G. *atlanticum* were evaluated by two complementary methods: DPPH radical scavenging capacity and reducing power. The free radical scavenging activity of tannin crude extract is presented in Figure 1. The preparation was able to reduce the stable free radical DPPH to the yellow-colored 1,1-Diphenyl-2-picrylhydrazyl with an IC_{50} = 16,03±0,87 µg /mL.The extract exhibited a weaker free radical scavenging activity than the synthetic antioxidant agent BHT (IC_{50} = 6, 29±1, 12 µg /mL), Tannin crude extract of *G. atlanticum* is a powerful substance against ROS represented here by Diphenyl-2-picrylhydrazyl; this may be due to the higher contents of polyphenols in it, which can exhibit scavenging activity against the free radical. In the reducing power

assay, the antioxidant molecules convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}).

The results of this research showed that the reducing power of tannin crude extract of *G. atlanticu*m was less than all standard (Figure 2). The results show a strong reducing capacity with EC_{50} of 13, 81±0, 36 µg/mL*** against the BHA 9, 09±0, 21 µg/mL. The reducing ability was found to be concentration-dependent. This may be due to the increase in the reducers (polyphenols) which would have converted the Fe^{3+}/ferricyanide complex to the ferrous form.

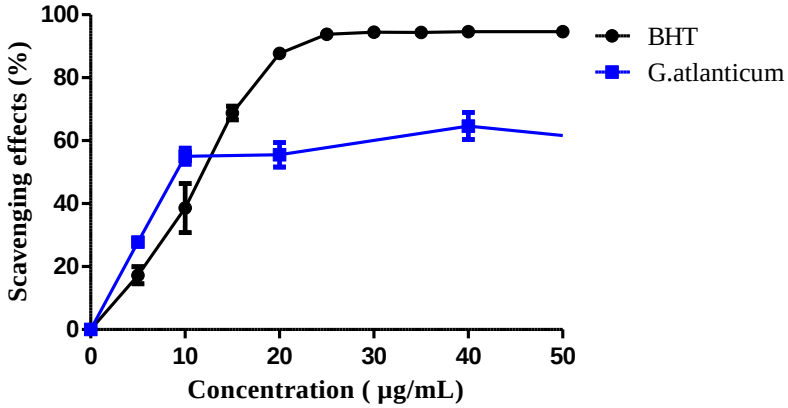

Figure 1. DPPH test of tannin crude extract of *G. atlanticum.*

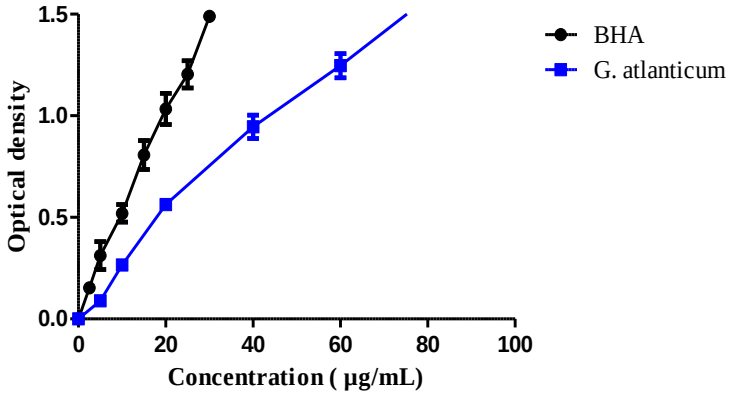

Figure 2. Reducing power activity of tannin crude extract of *G. atlanticum.*

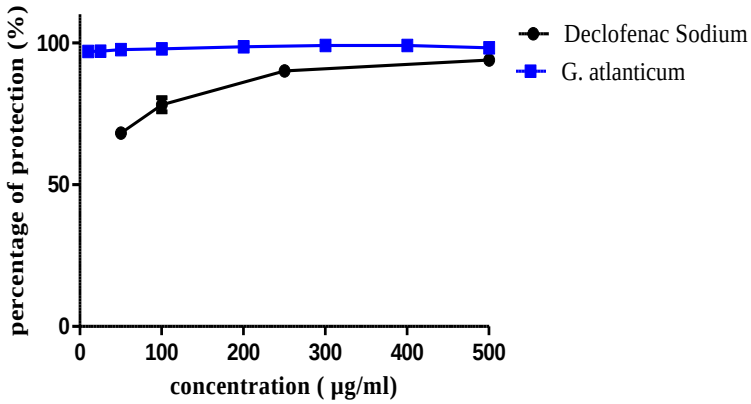

Figure 3. The percentage inhibition of hypotonicity induced haemolysis of HRBCs (%) of tannin crude extract of *G. atlanticum.*

To evaluate the anti-inflammatory activity of the prepared extract, human red blood cell membrane stabilization method was selected (this membrane is analogous to lysosomal membrane). The results (Figure 3) showed that the tested sample can perfectly protect the erythrocytes against hypotonicity induced haemolysis. Tannin crude extract of *G. atlanticum* inhibited hypotonicity induced HRBC membrane lysis by 96, 97±0, 21 %*** at a concentration of 10 µg/mL and still perfect until the concentration of 500 µg/mL (98, 18±0, 09 %** of protection). However, the standards make only 68, 13±1, 40 % of protection at a concentration of 50 µg/mL and increase to reach 93, 92±0, 95% at 500 µg/mL.

CONCLUSION

For the first time, tannin crude extract of *G. atlanticum* was valued to valorise the antioxidant and anti-inflammatory activities. The results show important sources of phenolic compounds, an excellent antioxidant extract and a perfect anti-inflammatory substance which may replace the NSAIDs. Further investigations concerning the chemical composition and toxicity are needed to deepen our study.

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