RESEARCH ARTICLE

Antioxidant and anti-inflammatory activities development of methanol extracts of two endemic species growth in the park TAZA JIJEL–Algeria (*Genista ferox Poiret* and *Genista ulicina Spatch*)

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Genista ferox Poiret and *Genista ulicina Spatch* are Algerian endemics species growth in the park TAZA JIJEL – Algeria valorized for the first time for their medicinal aspects. This experiment was carried out by the quantification of polyphenols and flavonoids, antioxidant and anti-inflammatory activities of methanol extracts of the two species. The antioxidant activities were evaluated by scavenging assays of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals and the reducing power and the anti-inflammatory activity by Human Red Blood Cell (HRBC) membrane stabilization method was used to evaluate the anti-inflammatory activity. The obtained results indicate an important anti-inflammatory activity at a very low concentration; encourage further biological investigation.

Key Words: *Antioxidant; Anti-inflammatory; Genista ferox; Genista ulicina*

INTRODUCTION

Located in the northeast of Algeria, TAZA National Park (3807 ha) was established in 1984 to protect the natural environment of the area by controlling hunting, wood cutting and road clearing. The area is a mixture of marine and terrestrial ecosystems including agricultural land, forests, rocky coasts, sandy beaches, grottos and springs. It is located about 30 km west

Of the province of JIJEL and extends along 9 km of coast [1]. It is distributed between municipalities of El-Aouana (837 hectares), Selma (1945 hectares) and Ziama-Mansouriah (1 025 hectares) [3].

Taza National Park (TNP) has the highest afforestation rate in Algeria. It is the only designated national area with pure and mixed oak (*Quercus canariensis, Quercus afares* and *Quercus suber*) which serve as habitats for a diversified and rich fauna of particular interest to preserve, including the Monkey Maggot, the Kabyle Nuthatch, red fox, raptors and water birds [2]. The number of floristic taxa counted in the TNP is about 420 species and subspecies of 258 genera and 71 botanical families of vascular plants (phanerogams and vascular cryptogams). The phanerophyte species represent about 13.57% of the park's flora, making 57 species. At the level of endemic species, we counted 52 taxa, represent a rate of 12.38% compared to the total species of the park. This endemic flora of TNP is distributed between Algerian (14 species), North African (22 species), Algerian-Moroccan (5 species), Algerian-Tunisian (11 species) [4].

Genista ferox Poiret is a North African endemic species, in the form of an evergreen shrub characterized by nearly glabrous calyx, deciduous in whole or in part on the pod, intersecting circularly above the base; this one 3-6 cm long. Oval leaflets 3-6 mm wide. Shrub of 1-3m, cheerful green. Old twigs transformed into huge thorns very vulnerable. Stipulated leaves, with stipules

Transformed into small prickles. This species prefers coastal and interior forests.

Genista ulicina Spatch is an Algerian endemic species known as an evergreen shrub characterized by slender thorns, very branchy, at least 10-15 divisions on the main stem. Orange flowers, quite large. Clusters flowering ending in leafy branches. Woollypod, prostrate, with rising beak, dark black. Found in brushwood [5].

The purpose of this investigation was to valorize the phytochemical and therapeutically sides of two endemic flora of TNP to expand the Algerian pharmacopeia.

MATERIALS AND METHODS

Plant material

The random sampling was used during the harvesting, the areal parts of *Genista ferox Poiret* and *Genista ulicina Spatch* were chosen haphazardly from TAZA National Park JIJEL – Algeria (36° 35' 16"- 36° 48' 12" north Latitude and between 5° 29' 07" and 5° 40' 11" Longitude East) Determined by Dr. Nouioua Wafa.

Preparation of methanol extracts

The areal parts of the chosen species were powdered and macerated in 80% methanol for 24, 48 and 72 hours, at the laboratory temperature (1:10 w/v, 10 g of dried herb). After maceration, the extracts were collected, filtered and evaporated to dryness under vacuum [6]. The dry extracts were stored at a temperature of -18 °C for later use.

Determination of total phenolic content

For total polyphenol determination, the Folin Ciocalteu method was used [7]. 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 absorbances were measured at 765 nm. 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).

Determination of total flavonoids contents

The flavonoids content was estimated by the Aluminum chloride solution according to the method described by Bahrun et al., (1996) [8]. Briefly, 1 mL of the methanol solution of the extracts was added to 1 mL of 2% AlCl3 in methanol. After 10 minutes, the absorbance was determined at 430 nm. Quercetins were used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/gE).

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DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hana to *et al.*, (1998) [9]. One milliliter of the extracts 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 measured at 517 nm. The antiradical activity was expressed as IC50 (micrograms per milliliter). The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%)=[(A0 – A1)/A0] ×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 [10]

Reducing power

The reducing power was determined according to the method of Oyaizu (1986) [11]. The extracts (0.5 –10 mg/mL) in methanol (2.5 mL) were 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 and the mixtures were centrifuged at 200 g for 10 minutes. The upper layers (5 mL) were mixed with 5 mL of deionized water and 1 mL of 1 mg/mL ferric chloride, and the absorbance was measured at 700 nm against a blank. Ascorbic acid was used as standard [12].

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 15 minutes thrice and washed with equal volume of normal saline each time. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

The mixtures 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 plant extracts 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% hemolysis.

The mixtures were incubated at 37°C for 30 minutes and centrifuged at 2500 rpm for 5 minutes.

The absorbance of hemoglobin content in the suspensions was estimated at 560 nm. The percentage of hemolysis of HRBC membrane can be calculated as follows:

Hemolysis (%)=(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] [13].

Statistical analysis

Results were expressed as mean ± standard deviation. Data were statistically analyzed using one-way ANOVA, Newman-Keuls Multiple Comparison and two-way ANOVA to determine whether there were any significant with the criterion of P values<0.05 between crud extracts of the two species and standards, using Graph pad prism 8 Demo Software.

RESULTS AND DISCUSSION

The yield, the quantity of total polyphenols and the flavonoids content were showed in Table 1:

TABLE 1

Yield, polyphenols and flavonoids quantification of extracts and standards

	Yield (%)	Polyphenols contents (mg EAG/gE)	Flavonoids contents (mg EQ/gE)
Genista ulicina	22.4	12.95 ± 0.61	10.30 ± 0.21
Genista ferox	22.1	22.44 ± .87	3.98 ± 0.68

Phenolic compounds derived from vegetable products are recognized to have antioxidant activities that act as auxiliaries in the functioning of the endogenous immune system [14]

Phenolic compounds have been shown to be responsible for the antioxidant activity of plant materials [15]. The two *Genestas* contain a low quantity of polyphenols but an important yield, which led us to suppose others group of antioxidant molecules.

DPPH is the most common antioxidant test which valorizes the power of the extract; the results are expressed in Figure 1:

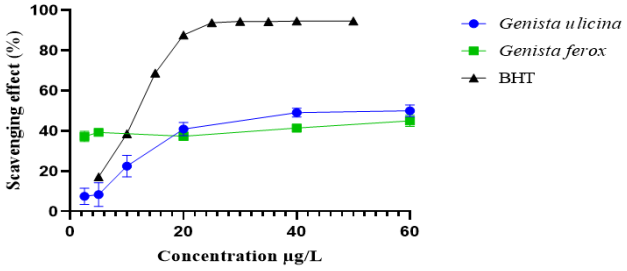

Figure 1) *DPPH test of Genista ulicina and Genista ferox crude extracts.*

The percentage of inhibitions were 74,67 ± 1,09% and 80,92 ± 1,02% for Genista ferox and Genista ulicina respectively, estimated important in comparison with BHT 87,96 ± 0,97% at the same concentration of 200 µg/mL. The values of IC50 of the extracts and standard are demonstrated in Table 2:

TABLE 2

IC50 of extracts and standards (DPPH test)

	IC_{50} (µg/mL)
Genista ulicina	43.68 ± 6.41***
Genista ferox	69.50 ± 3.58***
BHT	6.29 ± 1.12

indicates same concentration

The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts [16]. The extracts reduce and discolor the DPPH• radical due to their ability to yield hydrogen to the free radicals produced during peroxidation [17] [18]. *Genistas* crude extracts show a moderate to weak antioxidant power to reduce DPPH• molecules in comparison with BHT due to the low quantities of polyphenols.

Interestingly, Meza et al. (2020) [19] showed the effect of phytohormone stimulation on the total phenolic/flavonoids and the DPPH scavenging capacity *in vitro* produced from seeds, improving higher antioxidant activity and phenolic/flavonoid contents than other parts of the plant. This may explain the poor quantities and antioxidant weakness of the crudes extract of Genista ulicina and Genista ferox.

In the reducing power assay, the antioxidant compounds convert the oxidation form of iron (Fe+3) in ferric chloride to ferrous (Fe+2). The results are showed in Figure 2:

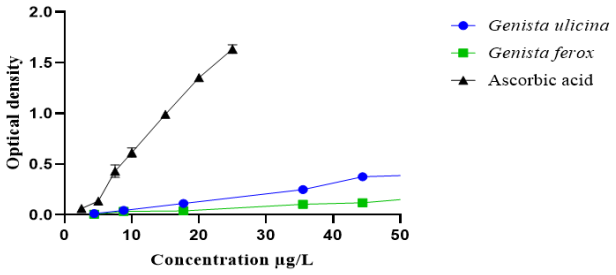

Figure 2) *Reducing power test of Genista ulicina and Genista ferox crude extracts.*

EC_{50} value (mg extract/mL) is the effective concentration at which the absorbance was 0.5 for reducing power, revels the power of extracts to reduce Fe+3 to Fe+2 demonstrated in Table 3:

TABLE 3

EC_{50} of extracts and standards (reducing power test)

		EC_{50} (µg/mL)
Genista ulicina	85.13	1.58***
Genista ferox	146.37	6.97***
BHT		6.29 ± 1.12

*** indicates same concentration

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [20]. *Genistas* crude extract results indicate a moderate to low capacity explained with the poor polyphenols contents in the crudes extracts.

The Stabilization of lysosome membrane is important in limiting the inflammatory response by preventing the release of lysosome constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further inflammation and damage on extracellular release [21]. The results are showed in Figure 3.

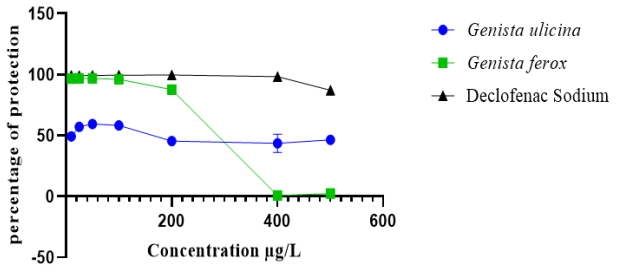

Figure 3) *The Human Red Blood Cell (HRBC) membrane stabilization test of Genista ulicina and Genista ferox crude extracts.*

Genista ferox crude extract gave a protection of 96, 54 ± 0, 29% at a concentration of 10 µg/mL against 99.31 ± 0.05% of Diclofenac sodium at the same concentration but this value declines significantly from 400 µg/mL to reach 0, 82 ± 1, 78%*** of protection against 98.178 ± 0.491% for Diclofenac sodium, however, Genista ulicina still giving a moderate value of protection whatever the concentration of the crud extract. The taxa of *Genista* L. are characterized by the presence of flavonoids and quinolizidine alkaloids, which are both important chemotaxonomic markers of the genus [22]. The action of flavonoids (kaempferol, quercetin, apigenin, apigenin 7-O-glucoside, luteolin) or even is flavonoids (genistein) [22], were observable at a very small concentration by protect the erythrocyte from the haemolysis but when concentration increases the quantity of others molecules (alkaloids and saponins) which act as haemolytic, which explain ours results. Further, Genista ulicina still in the medium case may explained by antagonism between the different groups of molecules.

CONCLUSION

The current study clearly indicates that the crude extracts of Genista ulicina and Genista ferox significantly affected by the exact vegetal organ of the areal part (polyphenols content and antioxidant activity) Genista ulicina indicate the best result Genista ferox in comparison with. However, Genista ferox demonstrate a powerful anti-inflammatory effect by protecting erythrocyte from haemolysis only at a small concentration. More In vivo biologicals activities are needed.

REFERENCES

1. Boubekri I, Djebar AB. Marine protected areas in Algeria: Future marine protected area of "Taza"(SW Mediterranean), continuing challenges and new opportunities facing an integrated coastal management. Ocean Coast Manage. 2016;130:277-89.
2. Atmiş E, Özden S, Lise W, et al. Urbanization pressures on the natural forests in Turkey: An overview. Urban Forestry & Urban Greening. 2007;6(2):83-92.
3. Chakour SC, Guedri SE. Sustainable management of artisanal fisheries in Algeria: The contribution of an empirical approach. Merit Res J Busin Manage. 2014;2(3):30-9.
4. Miara MD, Bendif H, Rebbas K, et al. Medicinal plants and their traditional uses in the highland region of Bordj Bou Arreridj (Northeast Algeria). J of Her Med. 2019;16:100262.
5. Quézel P. Analysis of the flora of Mediterranean and Saharan Africa. Ann Missouri Bot Gard. 1978;479-534.
6. Lakić NS, Mimica-Dukić NM, Isak JM, et al. Antioxidant properties of Galium verum L.(Rubiaceae) extracts. Cen Euro J Bio. Agro J. 2010;5(3):331-7.
7. Li WD, Wei C L, White P J. et al. High-amylose corn exhibits better antioxidant activity than typical and waxy genotypes. J Agri Food Chemis, 2007.55:291-298.
8. Bahorun T, Gressier B,Brunet C, et al. Oxygen species scavenging activity of phenolic extracts from hawthornfreshplant organs and pharmaceutical preparations. Arzneimittelforschung.1996;46(11):1086-9.
9. Hatano T, Kagawa H,Yasuhara T, et al. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull. 1988;36(6):2090-7.
10. Bettaieb I, Bourgou S, Sriti J, et al. Essential oils and fatty acids composition of Tunisian and Indian cumin (Cuminum cyminum L.) seeds: A comparative study. J Sci Food and Agri. 2011;91(11):2100-7.
11. Oyaizu M, Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. The Jap J Nutri Diet. 1986;44(6):307-15.
12. Huang SJ, Mau JL, Antioxidant properties of methanolic extracts from Agaricus blazei with various doses of γ-irradiation. LWT-Food Science and technology. 2006;39(7):707-16.
13. Volluri SS, Bammidi SR, Chippada SC, et al. *In vitro* antioxidant activity and estimation of total phenolic content in methanolic extract of Bacopa monniera. Rasayan J Chem. 2011;4(2):381-6.
14. Hakime-Silva RA,Vellosa JC, Khalil NM, et al. Chemical, enzymatic and cellular antioxidant activity studies of Agaricus blazei Murrill. Anais da Academia Brasileira de Ciencias. 2013;85(3):1073-82.
15. Rice-Evans CA, Miller NJ, Paganga G, et al. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933-56.
16. Koleva II, Van Beek TA, Linssen JP, et al. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis: An Intern J Plant Chem Biochem Techn. 2002;13(1):8-17.
17. Jeong SM, Kim SY, Kim DR, et al. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. J Agri Food Chem. 2004 ;52(11):3389-93.
18. Zheng CD, Li G, Li HQ, et al. DPPH-scavenging activities and structure-activity relationships of phenolic compounds. Nat Prod Commun. 2010;5(11):1934578X1000501112.

19. Meza A, Rojas P, Cely-Veloza W, et al. Variation of isoflavone content and DPPH• scavenging capacity of phytohormone-treated seedlings after *in vitro* germination of cape broom (Genista monspessulana). South Afri J Botany. 2020;130:64-74.

20. Chung YC, Chang CT, Chao WW, et al. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. J Agri Food Chem. 2002;50(8):2454-8.

21. Bakkali F, Averbeck S, Averbeck D, et al. Biological effects of essential oils–a review. Food Chem. Toxicol 2008;46(2):446-75.

22. Harborne JB. Chemosystematics of the Leguminosae. Flavonoid and isoflavonoid patterns in the tribe Genisteae. Phytochemistry. 196;8(8):1449-56.