INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 3, p. 178-183, 2017

RESEARCH PAPER OPEN ACCESS

Phytochemical study of three endemic brooms in Algeria: *Genista numidica* Spach, *Genista ferox* Poiret and *Genista tricuspidata* Desf

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Key words: *Genista numidica*, *Genista ferox*, *Genista tricuspidata*, Secondary metabolite, Antioxydant activity.

http://dx.doi.org/10.12692/ijb/11.3.178-183 Article published on September 27, 2017

Abstract

Genista numidica, Genista ferox and Genista tricuspidata are three endemic plants belonging to the family Fabaceae and which remain very little studied. The purpose of this work is to evaluate the antioxidant power of methanolic extracts of the flowers and the leaves of these species according to the DPPH free radical scavenging method while determining their total phenol, total flavonoids and tannin contents (the secondary metabolite), Flavonoids were estimated by the aluminum chloride (AlCl3) method, the total phenols were determined with Folin-Ciocalteu reagent and the tannins were estimated by the vanillin in acid medium method. The investigations showed that *G. numidica* is the most rich in polyphenols (113 ± 3.87mg and 79.6 ± 3.49 mg) of gallic acid equivalent and it's the most flavonoid-rich with 85.47 ± 1.51mg/g and 87.31mg/g of quercetin equivalent in flowers and leaves respectively. However, *G. ferox* was the most-rich in tannins in both leaves and flowers with 39.81 ± 0.32 mg/g and 44.36 mg /g of catechin equivalent respectively. *G. tricuspidata* showed a high level in the flowers extract (39.36 ± 0.32mg / g) and *G.numidica* gave 37.77 ± 0.64mg / g in the leaves extract. The antioxidant activity was the best in leaves and flowers extracts of *G. ferox* (IC50 = 0.50 ± 0.006 mg/ml and 0.59 ± 0.02mg /ml) as well as *G.numidica* leaf extract (0.55 ± 0.03 mg /ml). These results showed that this species would be suggested as a promising alternative source of the natural anti-oxidative phenolic compounds .

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Introduction

Algeria, because of the diversity of its bioclimates and the range of soils it contains, has a very interesting biodiversity. Algerian flora with its 3,000 species belonging to several botanical families, 15% of which are endemic, remains very little exploited phytochemically as well as pharmacologically. Among these plants, species belonging to the Genista genus, which alone contains 26 species and subspecies in Algeria, 11 of which are endemic (Quezel and Santa,1962 and 1963).

Phytochemical studies carried out on the Genista genus have permitted to isolate alkaloids (Pistelli, 2001 and Kirch, 1995**)**, as well as phenolic compounds, in particular flavonoids and isoflavonoids (Bolland, 1998 and Giachi, 2002**)**. This work is a part of the research and the development of bioactive substances such as natural substances with antioxidant activity which are of interest in the field of bio-pharmacology.

The objective of this work is to determine the levels of polyphenols, flavonoids and tannins of the methanolic extracts of the leaves and flowers of 03 Genista endemic species: *Genista numidica*, *Genista ferox* and *Genista tricuspidata* and also to evaluate, i*n vitro*, the antioxidant activity of the methanolic extracts in the three species according to the DPPH free radical scavenging

Materials and methods

Plant material

The aerial parts of *G. numidica* and *G. ferox* were harvested during the flowering period (April 2014) in the Cape of Guard zone (Annaba) and the aerial part of *G. tricuspidata* was harvested in the Berbessa area (Tipaza) in Algeria (Table 01).

Preparation of the methanol extract

The flowers and leaves of *G. numidica*, *G. ferox* and *G. tricuspidata* previously cleaned and crushed are macerated in methanol (2 g in 200 ml) with gentle stirring for 24 hours at room temperature. The alcohol extract is recovered after filtering the mixture with a filter paper; the methanol is removed from the filtrate by evaporation under reduced pressure in a rotavapor (BÜCHI). A crude extract was thus obtained.

Extraction yield

The extraction yield was calculated by the formula (*Falleh et al., 2008*):

R (%) = 100 Mext / Mech. Where: R is the yield in%; Mext is the mass of the extract after evaporation of the solvent in mg and Mech is the dry mass of the plant sample in mg.

Determination of total phenolic content (TPC)

The amount of total phenolic in all extracts, was determined with Folin-Ciocalteu reagent (Singleton *et al.*,1999).5 mg of samples or a standard solution of gallic acid were weighed and dissolved in 5 ml of ethanol. A volume of 0.5 ml of Folin-Ciocalteu reagent (previously diluted 2 fold with distilled water) was added into test tube containing samples and standard at room temperature for 5 min. 2.5 ml of sodium carbonate (20%) were added and left at room temperature around 90 minutes. The absorbance of mixture was evaluated at 765nm using a UV-Vis spectrophotometer. The TPC was expressed as gallic acid equivalent (GAE) in mg/g of extract (mg QE/g extract) based on the calibration curve.

Determination of total flavonoid content (TFC)

Total flavonoid content was estimated by the aluminum chloride (AlCl3) method (Ordon *et al.*,2006). 5 mg of samples or a standard solution of quercetin were weighed and dissolved in 5 ml of methanol. To 2 ml of sample, 2 ml of 2% AlCl3 ethanol solution was added. After one hour, at room temperature, the absorbance was measured at 430 nm. TFC was calculated as quercetin equivalent in mg/g of extract (mg QE/g extract) based on the calibration curve.

Determination of tannin content

The condensed tannins are determined by the method to the vanillin in acid medium (Julkunen-Titto,1985). A volume of 50 µl of the raw extract is added in 1500 µl of the solution vanillin/methanol (4 % m/v) then mixed by means of a whirlpool. Then, 750 µl some concentrated hydrochloric acid (HCl) is added.

The obtained mixture is let react to the hanging ambient temperature 20 min. The absorbance is measured in 550 nm against a white. A curve of calibration is realized in parallel in the same operating conditions by using of the catechin as the positive control. The results are expressed in equivalent (mg) of the catechin of the dry plant material (mg EC/g).

DPPH radical scavenging activity

To study the anti-radical activity of the various extracts, we opted for the method which uses the DPPH (diphényl picryl-hydrazyl) as a relatively stable free radical, according to the protocol described by (Brand-Williams,1995) .In this test antioxidants reduce the diphényl picryl-hydrazyl having a purple color in a yellow compound, the intensity of the color of which is inversely proportional to the capacity of the present antioxidants in the environment(middle) to give protons (Sanchez-Moreno ,2002). Briefly, 100 µl solutions of extracts were added to 2 ml DPPH (2,4mg prepared in 100ml methanol). At the same time (in parallel), a negative control is prepared by mixing 100 µl of methanol with 2ml of the méthanolic solution of DPPH. The reading of the absorbance is made against a white.

The reading of the absorbance is made against a white prepared for every concentration in 517nm after 30 min of incubation for the darkness and at room temperature. The positive control is represented by a solution of a standard antioxidant; the ascorbic acid and BHT the absorbance of which was measured in the same conditions as the samples and for every concentration, the test is repeated 3fois. The anti-radical activity is estimated according to the equation below:

% radical activity = [(Abs controls- Abs sample/ Abs controls] x100.

Where: Abs control is the absorbance of blank; Abs sample is the absorbance of the sample.

Results and discussions

Extraction yield

It is apparent from the observation of the extraction yields (Table 02) that the best yield was that of *G.numidica* (20% leaves) followed by that of *G. ferox* 17% (leaves and flowers) whereas *G.tricuspidata* gave the lowest yield 5% (leaves). The yield depends on the geographical origin of the plant, the season of harvest, method and conditions of the extraction. It is only relative (Benhamou, 2011).

Table 1. Ecological characteristics of sampling site.

Species	*G. numidica*		*G. ferox*		*G. tricuspidata*	
Organs	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
yield %	20	10	17	17	5	10

Table 2. Percentage yield of extractions in the 03 brooms.

Site	Latitude	Longitude	Altitude	Texture of substrate
Annaba	36°57'	7°46'	171 m	Sandy loam
Tipaza	36°37'	2°45'	392m	Sandy loam

Polyphenols content

The results in (Fig.1) show that *G. numidica* is the most rich in polyphenols for flowers and leaves (113 ± 3.87 mg GAE/g and 79.6 ± 3.49mgGAE/g), respectively, compared with *G. ferox* (86.66 ± 4 , 10mg GAE/g and 65 ± 1.74mg GAE/g) and *G.tricuspidata* (53.06 ± 0.64 mg GAE/g and 51 ± 0.72mg GAE / g).

Flavonoids content

Based on the results of the quantitative analysis of flavonoids (Fig 2); *G. numidica* was found to be the most flavonoid-rich with 85.47 ± 1.51mg QE/G and 87.31 mg QE/g, respectively, in flowers and leaves, followed by *G. ferox* (61.90 ± 1, 46mg QE/g and 56.06 ± 1.45 mg QE/g) and finally *G. tricuspidata* with the lowest content (44.91 ± 0.32 mg QE/g and 44.70 ± 1.14 mg QE/g), respectively.

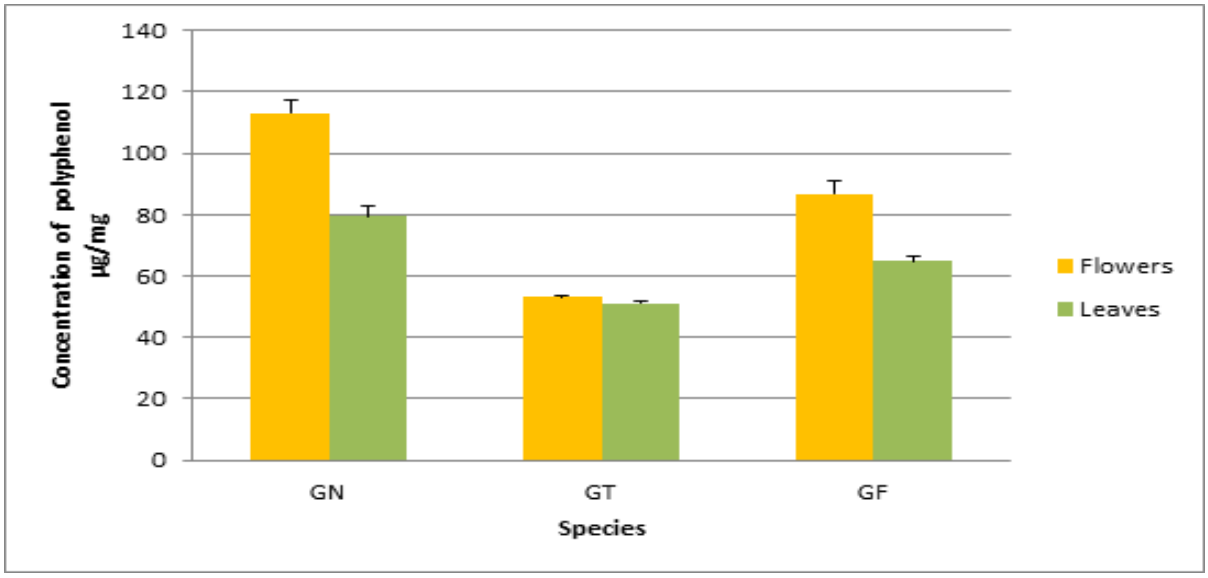

Fig. 1. Total polyphenol content in the 03 species (mg GAE/g).

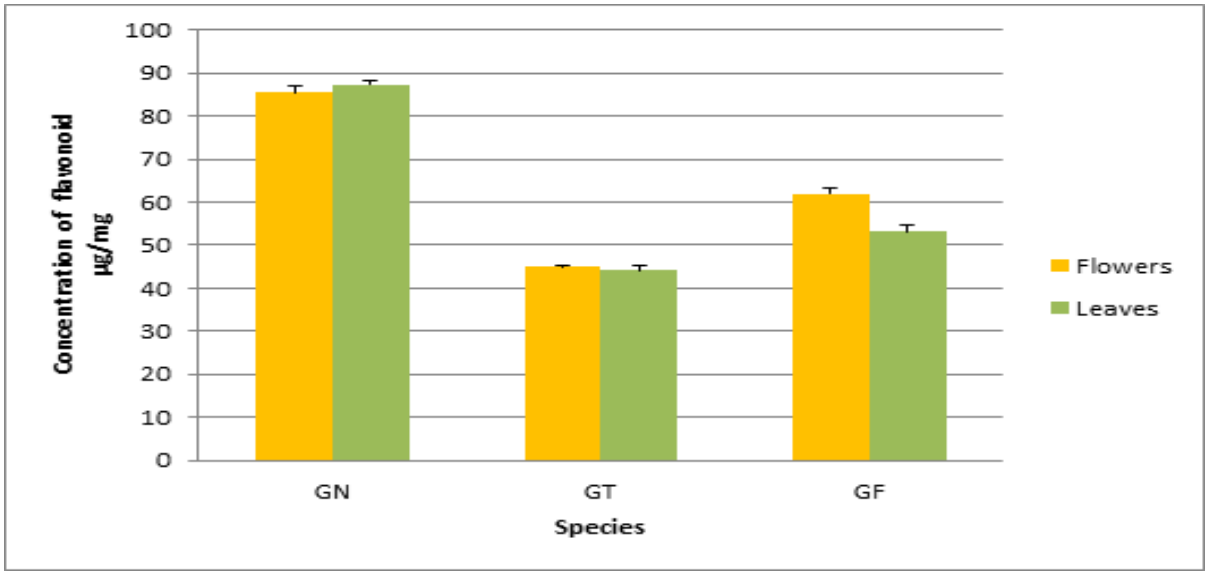

Fig. 2. Flavonoid content in the 03 species (mg QE / g).

Tannin content

The quantitative analysis of the tannins of the 03 species (Fig. 3) gave the highest levels in *G. ferox* both in leaves and in flowers with 39.81 ± 0.32 mg EC / g and 44.36 mg EC respectively / g. For *G. tricuspidata* the levels were respectively 30.27mg CE / g and 39.36 ± 0.32 mg CE/g and finally, for *G. numidica* the tannin contents were 37.77 ± 0.64mg CE / g and 30.18 ± 0.83mg CE/g respectively.

Antioxidant activity

The study of the antioxidant activity of the extracts of Genista species according to the DPPH free radical scavenging method (Fig. 4) showed that the IC 50 value of the ascorbic acid was equal to 0.51 + 0.004 mg/Ml and the IC50 value of BHT was 0.17 ± 0.02 mg/ml. By comparing the IC50 of our extracts with those of ascorbic acid and BHT, we observed a very important antioxidant activity of leaf extracts and flowers in *G. ferox* (IC50 = 0.50 ± 0.006 mg/ml and 0.59 ± 0.02mg/ml) as well as *G. numidica* leaf extract (0.55 ± 0.03 mg / ml).

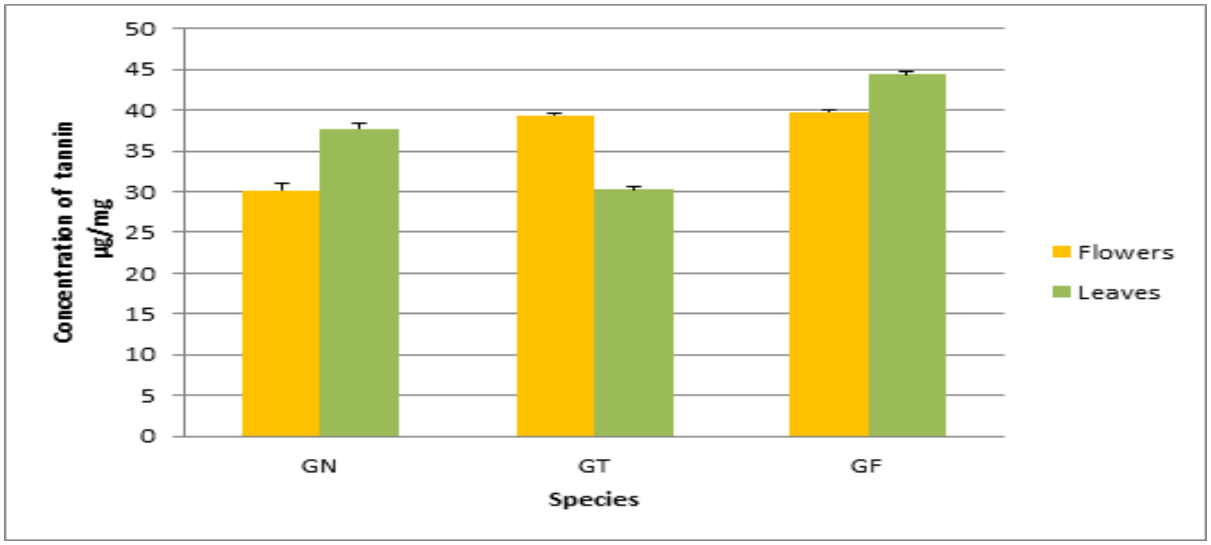

Fig. 3. Tannin content of the 03 species (mg CE / g).

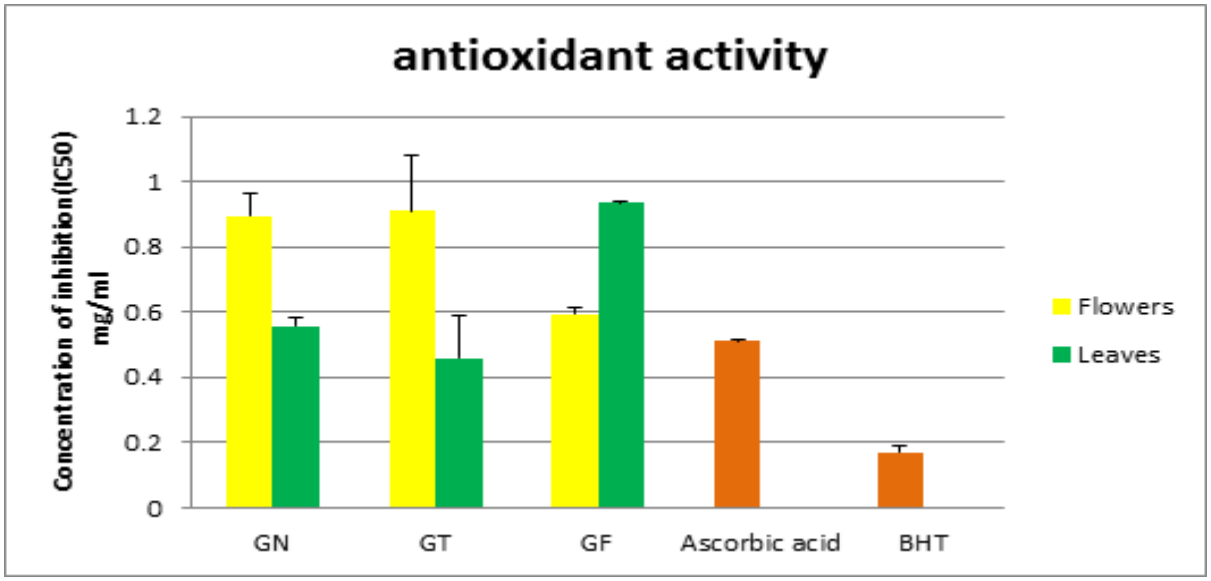

Fig. 4. Concentration of inhibition IC50 of the 03 species and of the reference antioxidants.

In contrast, extracts of leaves and flowers of *G. tricuspidata* (0.95 ± 0, 13 and 0.93 ± 0.16mg/ml) and *G.numidica* flowers (0.89 ± 0.06mg/ml) showed a very modest antioxidant activity. Few studies have been devoted to the antioxidant power of the 03 species of Genista, but we can note that *G. ferox* and *G. numidica* showed a good antioxidant activity. This is due to their richness in polyphenols and flavonoids. Many attempts to explain the structure activity relationship of some phenols have been reported in the literature (Ghasemzadeh A., *et al* 2011). (Halliwell,B and Guttridge,J 1999) reported that the power in the anti-oxidation process results first from the ability to prevent the anti-oxidation of free radical mediates oxidation of the substrates in low concentration and second that the resulting radical after scavenging must be stable.

Conclusion

The results obtained showed that the methanolic extracts of the leaves and the flowers of *G. ferox* and *G.numidica* yielded the best levels of polyphenols and flavonoids, which generated a good antioxidant activity. The antioxidant effect could prevent several diseases, so it would be interesting to test them *in vivo* to correlate the results observed in both cases. This work can also complement the already existing database of these three endemic species, which are very little studied and which require further research.

Acknowledgments

Our thanks go to Me Guetari_Mouhamadi without whom, this work could not have been realized.

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