International Journal of Chemistry and Pharmaceutical Sciences

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R E S E A E C H A R T I C L E

Antioxidant and anti-inflammatory activities valorisation of methanol extract of two *Fabaceae (Genesta pseudo-pilosa* and *Spartium junceum* L*)* growth in East of Algeria

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A B S T R A C T

The role of medicinal plants in promoting the ability of human health to cope with the unpleasant and difficult situations is well documented from ancient times till date all over the world. The present study aims to investigate the *in-vitro* anti-inflammatory and antioxidant activities of methanol extract of two Fabaceae. The antioxidant activity was evaluated by DPPH and the reducing power essay and anti-inflammatory activity with the Human Red Blood Cell (HRBC) membrane stabilization method. The results confirm the role of the two species as promising free radical scavengers, potent antioxidants and anti-inflammatory agents.

Keywords: *Genista*, *Spartium*, DPPH, reducing power, HRBC.

A R T I C L E I N F O

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PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 21 January 2019, **Accepted** 28 February 2019, **Available Online** 27 March 2019

Citation: Nouioua Wafa. *Antioxidant and anti-inflammatory activities valorisation of methanol extract of two Fabaceae (Genesta pseudo-pilosa and Spartium junceum L) growth in East of Algeria*. *Int. J. Chem, Pharm, Sci.*, 2019, 7(3): 60-63.

C O N T E N T S

1. Introduction

Leguminosae is the third largest family of flowering plants after Orchidaceae and Compositae, with approximately 650 genera and 18000 species [1]. The family Leguminosae is divided into three sub-families [2]: Mimosidae, Caesalpinioideae and Faboideae. Due to the large size of these sub-families they are now treated as independent families and named Mimoaceae, Caesalpiniaceae and Fabaceae [3]. Fabaceae, commonly known as the legume pea or bean family is a large economically important family of flowering plants [4]. The plant group is the third largest land plant family that comprised approximately 730 genera and 19400 species with a broad distribution and widely recognised importance. Many genera in this family are extremely widespread, while others are endemic to single countries [5]. One of the most important genera is *Genista* L. is a large genus of spiny and nonspiny shrubs centred in

the Mediterranean region. [6]. The genus *Genista* L. includes around 200 shrubby taxa, which are mainly distributed in the Mediterranean and nearest regions. The distribution of the genus stretches up to NW Europe, while in the south it outlines the boundaries of the Mediterranean region in Northern Africa. The highest concentration of species is found in the Iberian Peninsula. This genus is also widespread in western and central Europe, extending out to the South- East of Russia, into Turkey, Syria and the Caucasus [7] [8], divided into three subgenera and ten sections [6]: *Genista*, *Spartocarpus*, and *Phyllobotrys* [9]. Spanish Broom (*Spartium junceum* L.) is a small shrub available in Mediterranean countries, where it grows spontaneously [10]. Its habitat is the Mediterranean area of the South Europe, southwest Asia and North West Africa. In Italy, in the Mediterranean area of olive groves, it climbs the altitude of 975 meters. In Turkey, Syria and Palestine, it reaches altitudes of 1,700 meters. It is regarded weed in the USA and New Zealand with a tendency to eradicate it in order to save indigenous plants [11]. Due to their economics importance, we investigate the antioxidant and anti-inflammatory activities to enrich the Algerian pharmacopeia.

2. Experimental

Plant material: *Genista pseudo-pilosa* Batt was harvested from the massif of Boutaleb (X1 E: 5° 30’ 2.46” Y1 N: 35° 44’ 41.74"). *S. junceum* L from (X N: 36° 35' 16" Y E: 5° 29' 07") in May 2017.

Preparation of methanol extracts

The areal parts of the two species were powdered and macerated in 80 % methanol for 24, 48 and 72 h, 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 [12]. The dry extracts were stored at a temperature of -18 °C for later use.

Determination of Total Phenolic Content

For total polyphenol determination, the Foline Ciocalteu method was used [13]. 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 min 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 min 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 in our extracts were estimated by the Aluminium chloride solution according to the method described by Bahorun *et al*., (1996) [14]. Briefly, 1 mL of the methanol solution of the extracts was added to 1 mL of 2 % $AlCl_3$ in methanol. After 10 min, the absorbances were determined at 430 nm. Quercetin was used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/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 Hanato *et al*., (1998) [15]. One millilitre of the extracts at different concentrations was added to 0.5 mL of DPPH-methanol solution. The mixtures were shaken vigorously and left standing at the laboratory temperature for 30 min in the dark. The absorbances of the resulting solutions were 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:

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

Where: A_0: the absorbance of the control at 30 min

A_1: is the absorbance of the sample at 30 min. Butylated hydroxytoluene (BHT) was used as standard [16].

Reducing power

The reducing power was determined according to the method of Oyaizu (1986) [17]. 2,5 mL of the extracts were mixed with 2.5 mL of sodium phosphate buffer (pH 6.6 ; 200 mmol/L) and 2.5 mL of potassium ferricyanide (10 mg/mL). The mixtures were incubated at 50 °C for 20 min. After cooling, 2.5 mL of trichloroacetic acid (100 mg/mL) were added; the mixtures were centrifuged at 200g for 10 min. The upper layer (5 mL) was 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. 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. Ascorbic acid was used as a reference standard [18].

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 minthrice 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 principle involved here was stabilization of human red blood cell membrane by hypo-tonicity induced membrane lysis. The mixtures contain 1mL 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 % haemolysis.

The mixtures were incubated at 37 °C for 30 min and centrifuged at 2500 rpm for 5 min. 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] [19].

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analysed using one-way ANOVA and Newman-Keuls Multiple Comparison to determine whether there were any significant with the criterion of P values < 0.05 between methanol extracts of the two species and standards, using Graphpad prism 5 Demo Software.

3. Results and Discussions

The content of phenolic compounds was 14,45±0,55 mg EAG/GE for *G. pseudo-pilosa* and 8,61±0,36 mg EAG/GE for *S. junceum*. However, the total flavonoid content of extracts were 8,27±1,14 mg EQ/GE for *G. pseudo-pilosa* and 4,11±0,12 mg EQ/GE for *S. junceum*. DPPH radical scavenging activity assay is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. This assay measures the ability of a sample to donate hydrogen to DPPH radical. The scavenging effect of the extracts is demonstrated in figure 1:

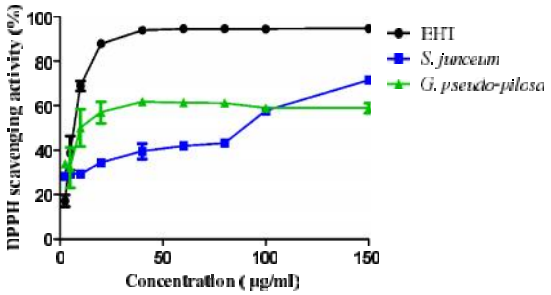

Figure 1: DPPH scavenging effect of extract of two Fabaceae *(G. pseudo-pilosa* and *S. junceum*

The best IC_{50} was observed in methanolic extract of *G. pseudo-pilosa* 19,35±9,16 µg/mL*, compared to the extract of *S. junceum* was 81,46±0,41 µg/mL** still weaker than BHT 6,74±1,06 µg/mL.

This difference of radical scavenging activity is attributed to the difference in phenolic and flavonoid content. Indeed, phenolic compounds, especially flavonoids are recognised as potentially antioxidant substances with the ability to scavenge free radical species. Fe^{+3} reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties. Figure 2 show the reducing power of the used extract.

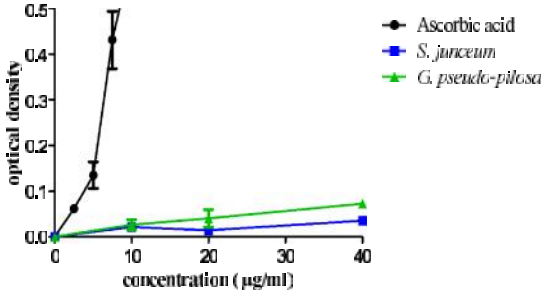

Figure 2: reducing power activity of two Fabaceae *(G. pseudo-pilosa* and *S. junceum*

The extracts exhibited less potent reducing power when compared to BHT: 8,46±0,09 µg/mL, at all tested concentrations. These findings reveal that the favourable reducing power observed in *G. pseudo-pilosa* 399,39±12,87 µg/mL*** flowed by *S. junceum* 497,46±2,16 µg/mL***. A relationship between Fe^{3+} reducing activity and total phenol content has been reported in the literature [20]. Figure 3 demonstrate *in-vitro* anti-inflammatory activity (membrane stabilization method) of the extrcts:

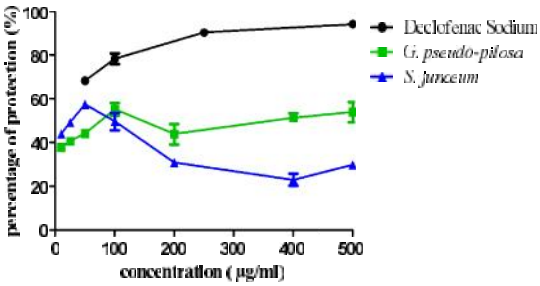

Figure 3: Protein denaturation inhibition power of standards and two Fabaceae (*Genista pseudo-pilosa* and *S. junceum*

The maximum of protection was observed at 50 µg/mL in *S. junceum* extract (57,37± 0,52 %) and 100 µg/mL (55,08±2,83 %) for *G. pseudo-pilosa* extract. These results were compared with standard diclofenac which make 68,13±1,40 % at 50 µg/mL and 78,14±2,50 % at 100 µg/mL. The statistical analysis of the serial data of the two species compared with the standard give a very high significant difference. However, the two species were important anti-inflammatory agents. The plant extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of RBC membrane. The RBC membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is play an important role in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [21].

4. Conclusion

The results show that extract of *G. pseudo-pilosa* have strong antioxydante activity then *S. junceum*, *however*, in anti-inflammatory activities the methanol extract *of S. junceum* show better haemolysis protection then the extract *of G. pseudo-pilosa*. Further studies are recommended to isolate the active molecules responsible for these activities.

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