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Phenolics compounds and antioxidant activities of *Anthyllis sericea* Lag. methanolic extracts, an endemic plant species grown in the South of Tunisia

Selmi. A[1*], Perez-perez. J. M[2], Bouzoumita. A[1], Benmoussa. N[1], Triki. T[1] and Ferchichi. A[1]

[1] *Arid and Oases Cropping Laboratory- Arid Area Institute (IRA)- Medenine- Tunisia.*

[2] *Institut of Bioingenieria UMH - Campus of Elche- Spain.*

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ABSTRACT

Phenolic compounds are integral constituents of many plants, and they have attracted a great deal of scientific interest because of their health-promoting effects as antioxidants. Anthyllis sericea, a plant used for many purposes, is traditionally used as a diuretic. In this study, total phenolic, flavonoid and tannin contents extracted from leaves, shoots and roots of Anthyllis samples collected from the south of Tunisia were evaluated. The quantitative estimation showed that Anthyllis samples were rich in polyphenol. While the leaf extracts contained the highest levels of total phenolic content, the root extracts had the highest content of flavonoids and tannins. The levels of phenolic compounds found in different organs indicate that they may have a strong antioxidant effect. In the DPPH and FRAP assay, all extracts shown substantial antioxidant effects. These results suggest that the leaves might play an important role in protecting the human body against free radicals.

Key words: *Anthyllis sericea, polyphenol, DPPH, FRAP.*

INTRODUCTION

Saharan plants were considered a source of free radical scavenging molecules, such as flavonoids, carotenoids, tannins, saponins, and terpenoids, which are responsible of the antioxidant activities. The high content of these natural compounds has attracted a great deal of scientific research in these species because of their exploitation as health-promoting compounds for the pharmaceutical industry.

Anthyllis spp. includes more than 170 species within the Papillionaceae family, which are distributed in Europe and Northen Africa [1]. *Anthyllis sericea* is an aromatic plant naturally grown in sandly-loam or sandy areas of south-eastern Tunisia (Tataouine, BeniKhedeche, Dhahar…) and Spain. This perennial shrub reaches 40-50 cm of height, the leaves are small and hairless. The flowers appear in clusters with yellowish white color.

These species are known for their traditional uses in folk medicine as a treatment for inflammation and to accelerate wound healing [2]. The flowers are used in cosmetology to promote hair growth [3], and are used as a diuretic and to treat mouth and throat diseases and ulcers. The fresh and dried leaves are also commonly used for cattle feeding and as a helminthagogue for sheep.

Recently, several studies to determine the chemical compounds present in some species of *Anthyllis* spp. have been performed, which confirmed the presence of flavonoids, glycosides, quercetin, tannins and polyphenols [4].

In this study, we aimed to characterize the phytochemical composition and the antioxidant potential of methanolic extracts obtained from leaves, stems and roots of *Anthyllis sericea* plants collected from south- eastern areas of Tunisia. The phytochemical screening was evaluated by calorimetric techniques to determinate the total content of phenolic compounds and total condensed tannin content. The antioxidant activity of the extracts was determined by the DPPH assay and compared with that of the standard (ascorbic acid) assay.

***Corresponding author:**
Selmi. A
Arid and Oases Cropping Laboratory - Arid Area Institute (IRA) - Medenine – Tunisia. Tel : 00 21 69 79 538 25.
**E-Mail: selmi_ayet@yahoo.com*

MATERIALS AND METHODS

Plant material: Several *Anthyllis sericea* plants were collected from the Dhahar (32° 30' 00" N,9° 50' 00" W)and Tataouine (32°55′46″ N ,10°27′06″ W)region, in the Southern East of Tunisia, during the flowering period (April 2013).The plant samples were spread on the ground, in an open room protected from the direct sunlight. During drying time, plants were turned over to allow homogeneous drying. After drying for three weeks, leaves, stems and roots from a single plant were cut with a razor blade into small pieces which were then used for the extractions.

Sample preparation: Two g of the dried plant material were grounded and dissolved in 20 ml of a 1:1 (v/v) methanol: water solution. The mixture was continuously stirred for 6 hours in the dark and then filtered through a Whatmann paper (3mm). The extract obtained was centrifuged (1536 *g*) for 20 minutes at 25 °C and the methanolic supernatant was kept at 4°C in the dark until the analysis.

Determination of total phenolic compounds: The amount of total phenolic compounds was determined using Folin–Cicalteu reagent, as described by Singleton and Rossi (1965) [5]. Briefly, 1-ml aliquots of the methanolic extracts were assayed with 250 l of the Folin reagent and 500 l of 20% (w/v) sodium carbonate. The mixture was vortexed and diluted with distilled water to a final volume of 5 ml. After incubation for 30 min at room temperature in the dark, the absorbance was determined at 765 nm. Three measurements were performed on each sample. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (DW), according to the equation: Absorbance = 0.0039 C; R^2 = 0.9988; where C refers to concentration.

Determination of total flavonoids: The total flavonoids were measured by the colorimetric assay developed by Zhishen and al, (1999) [6]. An aliquot (1ml) of appropriately diluted samples or standard methanolic solution of catechin (at different concentrations) was added to 10 ml volumetric flask containing 4 ml double distilled H_2O. At t = 0, 0.3 ml of sodium nitrite (5 %) was added to the flask. After five min 0.3 ml of $AlCl_3$ (10 %) was added. At six min, 2 ml of potassium (1M) was added to the mixture. Immediately, the reaction flask was diluted to higher volume (10 ml) with the addition of double distilled H_2O and thoroughly mixed. Absorbance of the mixture –pink color – was determined at

510 nm and compared to control (distilled H_2O). All samples were analyzed in triplicate. The total flavonoid content of the different extracts was expressed as mg of catechin equivalents (CE) per g of dry weight of plant material (mg CE / g DW), according to the equation: Absorbance = 0.333 C + 0.008; R^2 = 0.9981.

Determination of condensed tannin content: Tannin levels were determined by a spectrophotometric method (Swain and Hillis, 1959; Oueslati and al, 2012) [7, 8]. An aliquot (2 ml) of a freshly prepared solution of vanillin in methanol (1 %) in 70 % sulfuric acid was added to 1 ml of suitably diluted samples (polyphenol extract corresponding to 1 g of dry plant material). The mixture was incubated at 20°C in a water bath and after exactly 15 min; the absorbance at 500 nm was measured in a spectrophotometer compared to control. All samples were analyzed in triplicate.

Determination of the antioxidant activity:

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay: The most commonly used method for the evaluation of antioxidant effects is by measuring the scavenging of the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH)stable free radical. The different samples of *Anthyllis sericea* were screened for their antioxidant effect using the procedure described by Mighri and al, (2010) and Braca and al, (2002) [9, 10] with slight modifications. Different concentrations of extracts and standard (ascorbic acid) were prepared by diluting an appropriate amount of distilled H_2O. The test was carried out with a volume of 50 µl of each prepared solution mixed with 2 ml of methanol and DPPH solution (4 mg of DPPH in 100 ml of methanol). After stirring the mixture with a vortex, the aliquot is placed at room temperature and in darkness for 30 min. Then, the absorbance of the mixture was determined at 517 nm and compared to control (methanol). The DPPH values of each sample were compared with the scavenging effect of the standard (Ascorbic acid).All samples were analyzed in triplicate. The DPPH scavenging effects of the samples, in %, were calculated according to this equation:

DPPH scavenging effects (%) = [(A0 – (A-AA)) / Ao] x 100

A0 is A517 of DPPH without a sample (control). A is A517 of a sample and DPPH. AA is the A517 of a sample without DPPH (blank).

Ferric reducing antioxidant power assay (FRAP): The reducing power assay was described by Wang and al, (2008) [11] and Oyaizu (1986) [12] with ascorbic Acid (AA) and tert-butyl-4-hydroxyanisole (BHA) used as the positive controls. First, 2,5ml of deionized water was added to a aliquot of *Anthyllis sericea* extracts mixed with phosphate buffer and potassium ferricyanide (2,5 ml; 0,2M and 2,5ml;1%). A 2,5ml aliquot of trichloro acetic acid (10%) was added to the mixture, after incubation for 20 min in 50°C. Then, the solution was centrifugated at 3000 rpm for 10 min. A mixture of distilled water and ferric chloride was added to 5 ml of the previously supernatant and were be incubated at room temperature, in the dark, for 30 min.

The absorbance of this assay was measured at 700 nm. Samples of the assay were prepared in triplicate.

Table No. 1: Different yields (%) of methanolic crude extracts.

	Polyphenols	Flavonoids	Tannins
Leaves	16.60	1.20	4.20
Stems	10	1.05	1.2
Roots	14.7	1.20	2.12

Table No. 2: Different crude extracts showing Total phenolic, Flavonoid and Tannin contents.

	Polyphenols contents	Flavonoids contents	Tannins contents
Leaves	55.80 ±0.0	1.3+0.05	4.81± 0.063
Stems	7.1±0.02	1.2±0.013	6.12± 0.02
Roots	14.44±0.15	0.7±0.02	1.2± 0.03

Values are mean ± S.D of three replicates

Table No. 3: Different crude extracts showing antioxidant activity using DPPH activity, FRAP assay and IC values.

	DPPH radical scavenging activity	IC 50	Ferric reducing antioxidant power
Leaves	290.24±0.04	2.17±0.04	0.7± 0.01
Stems	202.43±0.02	4.81±0.07	0.12± 0.02
Roots	114.63±0.02	11.02±0.01	0.21± 0.01

Values are mean ± S.D of three replicates

RESULTS AND DISCUSSIONS

The percentage yield of extraction of A. sericea: Extraction results for different parts (leaves, stems and roots) of *Anthyllis sericea* are shown in table 1. According to the results obtained, the yields (w/w) of total polyphenol extracts (leaves: 16.60 %; stems: 10 % and roots:14.70%) were higher than flavonoid extracts(leaves: 1.20 %; stems: 1.05 % and roots: 1.20 %) and tannins extracts (leaves: 4.20 %; roots: 2.12% and stems: 1.2%).These results showed that polyphenol extracts contain flavonoids, tannins and others chemical compounds like: phenolic acids, coumarins have solubility in extraction solvent used. Moreover, the yields of methanolic extracts from leaves of *A. sericea* were found much higher than found in others parts of plant. Finally, the presence of phenolic compounds (flavonoids, tannins, others phenolic compounds) in different parts of *A. sericea*, indicates that this plant may have the ability as an antioxidant agent.

Total phenolic, flavonoid and condensed tannins in leaves, steems and roots of A. sericea

Several recent studies showed that phenolic constitute the main powerful antioxidant compounds [13]. For that, total phenolic, flavonoid contents of methanol– water (50/50, v/v) extracts from leaves, stems and roots of *A.sericea* were assessed.

Principal results showed in table 2 that *A. sericea* extracts exhibited an important amount of polyphenol content (14.44-55.80mg/g), followed by proanthocyannidins (4.81–6.12mg/g) and flavonoids (1.2–1.3mg/g). The leaves extract of *A. sericea* contained the highest total phenolic content of 55.8 (mg/g). Roots extract of *A. sericea* had comparatively lower than that total phenolic content of 7, 1 mg/g.

Although phenolic compounds are found in all plants and plant parts, their quantitative distribution varies between different organs in a plant [14]. *A. sericea* leaves extract was found to contain the highest flavonoid content (1.3 mg /g), when compared to that of *A. sericea* roots (1.2 mg/g).Flavonoids have been reported to be responsible for antioxidant activity [15].

A. sericea roots extract was contained the highest level of condensed tannins (6.12mg/g) than leaves extract that have (4.81mg/g). The antioxidant, anti–inflammatory, antifungal and healing properties of some plant extracts have been attributed to the presence of tannins [16].

The results of the study of Ghalem et al. (2012) [17] showed that the leaves methanolic extract of *A. vulneraria* from Algeria, contained the total phenolic content of 185.00 mg of Gallic acid equivalents /g DW. We conclude that the leaves of *A. sericea*

from southern of Tunisia contain less phenolic compound than those of *A. vulneraria* from Algeria.

Phenols, mainly anthocyanins are chemical compounds with high antioxidatif effects that play an important role in the adaptation of plants to abiotic stress factors [18-20], *Anthyllis sericea* leaves have a high-test level of flavonoids (1.3 mg/g) than that found in *A.vulneraria* from Algeria (0.11mg/g), this result show that leaves of *Anthyllis sericea* from Tunisia have been reported to be responsible for antioxidant activity.

The role of anthocyanins in leaves is still under debate. The basic question is whether they are directly involved in stress responses or just assist in plant defense [21]. For instance, anthocyanins and carotenoids are suggested to protect chloroplasts from excess [22]. Our data demonstrate that anthocyanins accumulation from leaves stems and roots increased under water deficit (different parts of plant are collected from a stress period) conditions. This increase suggests that anthocyanins can be involved in photo protection under direct drought stress [23-25].

Antioxidant Activity:

The content of total phenolic expressed as GAE (Gallic acid equivalent) in mg/mL of plant extract, varied from 290, 24 mg GAE /mL of leaves extract to 114, 63 mg GAE/mL of stems extract. The phenolic compounds have been reported to be significantly associated with the antioxidant activity of plant and food extracts mainly because of their redox properties, allowing them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers, and metal chelators [26]. The DPPH radical is stable and free, also used as a substrate to evaluate the antioxidant activity of extracts of vegetables and medicinal plants. Antioxidants can scavenge the radical by hydrogen donation, which causes a decrease of DPPH absorbance at 517 nm. The concentration of sample at which the inhibition value in 50% is defined as the IC50 value. Thus, the lower IC50 value indicates higher antioxidant activity. The values varied from 2,17µl/mL DPPH solution for the leaves extract of *Anthyllis sericea* (The most active) to 11,02 µl /ml DPPH solution for the stems extract (the lowest active).

The ferric reducing antioxidant activity in leaves, stems and roots were presented in Table3. FRAP measures the ability of the extract to donate electron from (Fe^{2+}) form to (Fe^{3+}) form. The reducing power were be determined by measuring the formation of Perl's Prussian blue at 700 nm [27, 28]. In this assay, the color of this solution changes from yellow to blue color depending on the power of the antioxidant samples. A higher absorbance indicates a higher reducing ability. The reducing power of the sample extracts increased with increasing concentration. For the concentration, the reducing power of leaves (0,7mg/ml) was higher than that of stems (0,21mg/ml) and roots (0,12mg/ml). The results showed that the FRAP activity of leaves is the greatest among all other extracts studied.

Given these results, we can say that leaves of this plant show a high content of phenolic compounds with strong antioxidant activity.

CONCLUSIOS

Considering the results of the percentage yield of extraction (polyphenol, flavonoids and tannins) and total phenolic, flavonoid and tannin contents, we can conclude that leaves of *A. sericea* contained appreciably high level of total phenolic compounds. The most sample extracts (polyphenol, flavonoids and tannins) from this plant also exhibited high antioxidant activities.

These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antioxidant agent from *A. sericea* plant. This medicinal plant by in vitro researches can appear as interesting and promising and may be effective as potential sources of novel antioxidant drugs.

Further work could be realized on the identification, isolation of the specific compounds from the crude extracts which are responsible for the higher antioxidative action.

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