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EVALUATION OF ANTIOXIDANT POTENTIAL AND PHYTOCHEMICAL STUDIES OF ONONIS ANGUSTISSIMA L. (FABACEAE)

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ABSTRACT

Medicinal plants would be the best source to obtain variety of antioxidant compounds. Effectively, several studies have demonstrated the potential of plant products as antioxidants against various diseases. By their resistance to several stress factors and under extreme climatic conditions, Saharan plants could contain biomolecules potentially useful as antioxidants. *Ononis angustissi*ma is an endemic plant of the north of Algerian Sahara. The aerial part were used by local people for its hemostatic properties. According to literature, *Ononis angustissima* was previously little chemically studied. The originality of the present study consists in scrutinizing the phytochemical composition and evaluating the antioxidant activity of aerial parts of aqueous extract of *Ononis Angustissima* (AEO) in conditions corresponding to its traditional use. Phytochemical screening was realized to scrutinize the qualitative composition of (AEO) using precipitation and coloration reactions. The total phenolics, flavonoids, tannins and β carotene contents were determined by using spectrophotometric methods. Finally, AEO was submitted to antioxidant activities assays using 3 methods : DPPH, reducing power and β carotene bleaching tests. The obtained results show the presence of different metabolites in AEO such as, phénolics compounds, flavonoids, tannins, terpenoids, glycosides, steroids and saponins. In addition, the quantitative analysis shows a considerable total phenolics, tannins and flavonoids contents. In sum, it was

found that EAO possess a good antioxidant activity against bleaching of β carotene, which sustains its use by traditional healers. Its good antioxidant activity may be explained by the high occurence of particullary tannins. Thus, *Ononis Angustissima* could be candidate for a good source of antioxidant compounds.

KEYWORDS: phytochemical screening, antioxidant activity, DPPH test, reducing power, β carotene, flavonoids, tannins.

INTRODUCTION

Free radical is defined as unstable, highly reactive atom or molecule possessing unpaired electrons, which induces free radical damage.[1] Recently, increasing evidence highlights that overproduction of reactive oxygen species (ROS) and oxygen-derived free radicals may contribute to a variety of pathological effects (DNA damages, carcinogenesis and cellular degeneration) and induce many diseases including aging, cancer, atherosclerosis, diabetes and rheumatoid arthritis.[2, 3]

In order to reduce ROS-induced oxidative damage, synthetic drugs as BHT and BHA are used.[4] However, these chemicals are toxic and their risk to health has increased the demand for natural antioxidants. Therefore, new antioxidant drugs lacking those effects are being searched all over the world as alternatives to synthetic drugs.[5] In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs.[6] According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants.[7] Effectively, Several studies have demonstrated the potential of plant products as antioxidants against various diseases induced by free radicals; so, these plants could constitute a major source of new chemical compounds which may be characterized by medicinal and biological properties.[8]

By their resistance to several stress factors and under extreme climatic conditions, Saharan plants could constitute a reservoir of new natural, safe and effective biomolecules potentially useful as antioxidants.[9]

The *Fabaceae* are the third largest family of flowering plants in term of number. Among them, a special place may be given to *Ononis* genus. *Ononis* comprises 75 species in the Mediterranean region, Asia and Europe.[10] There are over 34 species that exist in Algeria.[11] The genus have been shown to possess antipyretic, antibiotic, analgesic, antimicrobial, anti-inflammatory, antiseptic, cytotoxic, antidiabetic and diuretic activities.[12, 13,14]

Ononis natrix which is similar to *Ononis Angustissima*, is a small plant known for its medicinal properties. The infusion of its roots has diuretic and antirheumatic properties and has been used for the treatment of certain disturbances of the urinary tract.[15] High content of resorcinol derivatives found in Natrix L. subsp. Natrix.[16]

*Ononis angustissi*ma Lam. is an endemic plant of the north of Algerian Sahara (*Guardaia, Bechar, Biskra, Boussaada and Beni Abbas*). [17] It has a yellow flower [18] Only Chehma and Djebar (2015) reported the traditional use by decoction of the aerial part of the species *Ononis angustissima* for its hemostatic properties.[19] According to literature, O. angustissima, the subject of this study, was previously little chemically studied. However, it has been found that its aerial parts are a good source of antioxidant polyphenols and flavonoids.[17,20]

Unfortunately, there are no reports on the chemical composition or antioxidant potential of the aqueous extract of *Ononis Angustissima*. So, in order to extend our knowledge on this species, for the first time we scrutinize the phytochemical composition and evaluate the antioxidant activity of aeriel parts of *Ononis Angustissima* in conditions corresponding to its traditional use (Decoction).

MATERIALS AND METHODS

Plant material. The aerial parts of the plant were harvested in the region of *Hadjeb*, west of *Biskra*, located at a longitude of 5° 36’ Est and latitude of 34°47’ North. The plant was collected during flowering stage in April 2015 and the biological tests were conducted one month later. Their identification was carried out by Pr. *LAOUER Houcine* of laboratory of development of natural biological resources (LVRBN) at the University of Setif. Voucher specimens were stored in the herbarium of departement of Biology of ENS Kouba Algiers, Algeria.

Drugs and Chemicals. 1,1-diphenyl-2-picryl-hydrazil (DPPH•), potassium ferricyanide (K3Fe3+(CN)6), trichloroacetic acid (TCA), ferric chloride (FeCl3), β-carotene, linoleic acid, Tween-40, Folin–Ciocalteu's reagent, ammonia solution (NH3), sulfuric acid (H2S04). Hydrocloric acid (HCl), picric acid, acetic anhydride, chloroform, Fehling's solutions A and B, sodium carbonate, Aluminum chloride (AlCl3) and bovine blood. Ethanol, methanol, acetone et hexane were obtained from *Merck* (*Darmstadt, Germany*). Gallic acid, Quercetin, Tannic acid, Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) are used as positive standards. Authentics standards were purchased from *Sigma-Aldrich, Fluka and Merck*. All other chemicals used were of analytical grade.

Extraction procedure. To obtain the aqueous extract of the plant, the aerial parts (leaves, flowers and stems) were well cleaned and then were dried away from moisture and sunlight for a month. Decoction was carried out according to the protocol described by Ferreira *et al.*, (2013).[21] with some modifications. Briefly, the dried plants were sprayed using a grinder, then 50 g of the plant powder are added to 500 ml of extraction solvent (water). The mixture was heated on a hot plate with continuous stirring at 30º-40ºC for 20 minutes. Then, the water extract was filtered through Whatman No. 4 filter paper and centrifuged at 3000 rpm for 15 min. Finally, the supernatant obtained is dehydrated by a lyophylisator (*Christ Gamma 2-16 LSC plus*) until a brown powder formed, which was kept at -4 degrees Celsius in opaque bottles until used.

Extract yield percentage. The extraction yield is a measure of the solvent's efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mass compared with the initial amount of whole plant. It is presented in percentage(%) by the formula given by Falleh *et al.*, (2008).[22] **Y (%) = 100 Mext / Msamp**.

Where in: Y is the % yield; Mext is the mass the extract after evaporation of the solvent in mg and the Msamp is dry mass of the organ sample in mg.

Phytochemical analysis. Phytochemical screening was performed to assess the qualitative and quantitative chemical composition of aqueous crude extract of *Ononis Angustissima* (AEO) using commonly employed precipitation and coloration reactions to identify secondary metabolites like phenolic compounds, flavonoids, alkaloids , tannins, steroids, terpenoids, glycosides and saponins . Whereas, to estimate the content of the major secondary

metabolites like phenolic, flavonoids, tannins and β carotene, spectrophotometric technics were used.

Qualitative analysis. The qualitative analysis were carried out using standard procedures quoted by Sofowora *et al.*, (1993) and Trease *et al.*, (1989).[23,24] The plant powder was taken in a test tube and distilled water was added to it such that plant powder soaked in it and shaken well. The solution then was filtered with the help of filter paper and was taken and used for further phytochemical qualitative analysis.

Screening for Phenols. To 1 ml of the extract, 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenols.[25]

Screening for flavonoids. For the confirmation of flavonoid in the selected plant, 0.5 g of AEO was added in a test tube and 10 ml of distilled water, 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract followed by addition of 1 ml concentrated H2S04. Indication of yellow color shows the presence of flavonoids.[26]

Screening for alkaloids. For the purpose of phytochemical analysis of the selected plant, 0.2 g of the aqueous extract sample was added in each test tube and 3 ml of hexane was mixed in it, shaken well and filtered. Then, 5 ml of 2% HCl was poured in a test tube having the mixture of plant extract and hexane. The test tube having the mixture was heated, then filtered and a few drops of picric acid in a mixture was added. Formation of yellow color precipitate indicates the presence of alkaloids.[26]

Screening for tannins. 1 ml of water extract was mixed with 10 ml of distilled water and filtred.Ferric chloride (FeCl3) reagent (3drops) was added to filtrate. A blue-black or green precipitate confirmed the presence of tannins.[27]

Screening for steroids. 1 ml of acetic anhydride was added twice to 0.5 g of aqueous extract with 2 ml H2S04. The colour changed from violet to blue or green in sample indicating the presence of steroids.[28]

Screening for terpenoids (Salkowski test). 5 ml of aqueous extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish

brown coloration of the interface was formed to show positive results for the presence of terpenoids.[29]

Screening for glycosides (Fehling reaction). 4 ml of the aqueous extract was placed in a test tube and a 5 ml mixture of equal volumes of Fehling's solutions A and B was added and boiled in a water bath for 5 minutes. It resulted in brick- red cuprous oxide precipitate that contains sugar.[30]

Screening for saponins (Bubble test). 5 ml of aqueous extract was shaken vigorously for 2 minutes. The appearance of foam that persisted for at least 15 min confirmed the presence of saponins.[31]

Quantitative analysis

Total phenolics determination. Total phenolic content was estimated by the Folin–Ciocalteu method.[32] 200 µl of different concentrations of sample were added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. After 4 minutes, 800 µl of saturated sodium carbonate (75 g/l) was added. After 2 hours of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0–200 mg/l) was used for the standard calibration curve. The results were expressed as mg of Gallic acid equivalent (GAE)/g of dry weight of *Ononis Angustissima*, and calculated as mean value ± SD (n = 3).

Flavonoid determination. Flavonoids content in the extract was estimated by the Aluminum chloride solution (colorimetric assay) according to the method described by Bahorun et al., (1996).[33] Briefly, 1 ml of the aqueous extract was added to 1 ml of 2% AlCl3 (in methanol). After 10 minutes, the absorbance was determined at 430 nm. Quercetin (0 – 20 mg/l) prepared in methanol was used as a standard. Results were expressed as mg of Quercetin equivalent (QuE)/g of dry weight of *Ononis Angustissima*, and calculated as mean value ± SD (n = 3).

Tannin determination. Tannins content was evaluated using the hemoglobin precipitation assay as described by Hagerman and Butler, (1989).[34] An aliquot of 0.5 ml of the aqueous extract was mixed with 0,5ml of hemolysis bovine blood to reach a final concentration of 1g/l, then the mixture was centrifuged at 480g for 20 minutes and the absorbance was measured at 578 nm . Tannic acid (0 – 600 mg/l) was used as a standard.

Results were expressed as mg of Tannic acid equivalent (TAE)/g of dry weight of *Ononis Angustissima*, and calculated as mean value ± SD (n = 3).

B carotene determination. The content of β carotene was determined by the spectrophotometric method of Nagata and Yamashita, (1992).[35] Briefly, 100 mg of aqueous extract of *Ononis Angustissima* was vigorously agitated with 10 ml of acetone-hexane (4: 6) for 1 min and then filtered through Whatman filter No 4. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm.

The content of β carotene was calculated according to the following equation : **β Carotene (mg / g) = 0,216A663 - 1,22A645 - 0,304A505 + 0,452A453.**

(A663, A645, A505 and A453 are the absorbance at 663nm, 645nm, 505nm and 453nm, respectively). The tests were performed in triplicate; the results were the mean values ± standard deviations, expressed in mg of β Carotene / g of extract.

Antioxidant activity

DPPH radical scavenging assay. The free radical scavenging activity of aqueous extract of *Ononis Angustissima* (AEO) was measured by DPPH• using the method of Que *et al*, (2006).[36] Breifly, 0.1 mmol/l solution of DPPH radical in ethanol was prepared and 2 ml of this solution was added to 2 ml of water solution containing different concentrations of EAOand BHT. After 30 minutes absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging of DPPH radical in percentage was calculated by the following equation : **Scavenging activity (%) = (1- A1/A0)100%.**

Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of EAO and BHT. BHT was used as positive controls. The inhibitory concentration of 50% of the activity of DPPH (**IC50**) of EAG was thereafter calculated from the equation that determines the percentage inhibition versus concentration of inhibitor. It was expressed as µg / ml and compared with that of BHT.

Reducing power assay. The reducing power was determined by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess Fe3+, as described by Oyaizu *et al*, (1986).[37] Briefly, 2.5 mL of different concentrations of aqueous extract (0.5–10 g/l) was mixed with 2.5 ml of 200 mmol/l of sodium phosphate buffer (pH

6.6) and 2.5 ml of potassium ferricyanide (10 mg/ml), and the mixture was incubated at 50 C° for 20 min. Then, 2.5 ml trichloroacetic acid(100 mg/ml) were added, the mixture was centrifuged at 200g for 10 minutes. The upper layer (5 ml) was mixed with 5ml of deionized water and 1ml of 1 mg/ml ferric chloride. Then, the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. **IC50** 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. BHT was used for comparison.[38]

β-carotene/linoleic acid bleaching assay. The ability of AEO to prevent the bleaching of ß carotene was determined as described by Koleva *et al*, (2006).[31] In brief, 0.5 mg b-carotene in 1 ml chloroform was mixed with 25 µl of linoleic acid and 200 µl of Tween-40. The chloroform was evaporated under vacuum at 45 °C, then 100 ml distilled water was added and the resulting mixture was vigorously stirred. The emulsion obtained was freshly prepared before each experiment. An aliquot (2.5 ml) of the β-carotene- linoleic acid emulsion was transferred to tubes containing 0.5 ml of the sample at different concentrations. The tubes were immediately placed in water bath and incubated at 50 °C for 2 hours. Thereafter, the absorbance of the sample was measured at 470 nm. A control consisted of 0.5 ml of distilled water instead of the sample solution. BHT and BHA were used as positive standards.

Statistical analysis. Results were expressed as the mean ± standard deviation. Statistical differences were assessed using one-way ANOVA to determine whether there were any significant ($P < 0.05$) differences between the aqueous extract of Génista Saharae and controls. Data were subjected to analysis using the Microsoft Excel 2013 and Graph pad prism 6.

RESULTS AND DISCUSSION

Extraction yield of plant material. Extraction yield (w/w) for aqueous decoction of aerial parts of *Ononis Angustissima* was found to be in order of 9,75 % in term of dry weight. The choice of water as solvent is not done randomly. Indeed, the major factors affecting the choice of solvent are quantity of phytochemicals to be extracted, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate diversity of different compounds extracted and

toxicity of the solvent in the bioassay process.[40, 41,42] In addition, water soluble phenolics have important significance as antioxidant compound.[43]

Previous work on the aqueous extract of *Genista Saharae* species (AEG) which belongs to the same family from that of *Ononis Angustissima (Fabaceae),* shows that the yield of aerial parts of EAG was in order of 10.33%,[44] which is similar to that obtained in the present study. This result is expected as long as the two plants belong to the same geographical area and the same family (*Fabaceae*).The efficiency of aqueous extraction increases with temperature because the high-temperature water causes disruption of cells which promote penetration and solubilization of the solvent molecules.[45]

Phytochemical analysis

Qualitative analysis. Qualitative analysis of aqueous extract from *Ononis Angustissima* is summarized in the **table 1**.

Table1. Qualitative analysis from *Ononis Angustissima (Fabaceae)*

Chemical groups	Aqueous extract
Phenols	+
Flavonoids	+
Tannins	+
Alkaloids	-
Steroids	+/-
Terpenoids	+
Glycosides	+/-
Saponins	+/-

+ = indicates presence of chemical groups; +/- = indicates traces of chemical groups.

The AEO showed a considerable occurrence of phytochemicals. According to Wadood *et al*, (2013), [26] these constituents are considered as active medecinal chemicals; Phenols, flavonoids, tannins and terpenoids are strongly present in aqueous extract, while steroids, glycosides and saponins show traces. By cons, alkaloids were absent in the aqueous extract of *Ononis Angustissima*.

Polyphenolic compounds such as phenolic acids, vitamins (atocopherols, ascorbic acid) and other substances is widely used as safe natural antioxidants.[46] These non-nutritive chemicals have protected the humans from oxidative stress related disorders.[47] Flavonoids comprise a large group of polyphenolic compounds that are characterized by a benzo-y-pyrone structure,

which is ubiquitous in vegetables and fruits. Besides their relevance in plants, flavonoids are important for human health because of their high pharmacological activities as radical scavengers.[48] They have been reported to possess a variety of biological activities including antiallergic, antidiabetic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic, hepatoprotective, and antioxidant activities.[49, 50,51] Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, hemorrhoids and diarrhea.[52] It has also been reported to posses wound healing properties through anti-inflammatory, analgesic[53] and antioxidant modalities.[54] Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity.[55] Several workers have reported the analgesic[56,57] antispasmodic and antibacterial properties of alkaloids.[58,59] Glycosides are known to lower the blood pressure according to many reports.[60] Terpenoids exhibit various important pharmacological activities, anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities.[61] Polysaccharides are well known to be the major structural components of mushrooms and to possess immunomodulatory and anti-inflammatory activities.[62,63] Polysaccharides isolated from different mushrooms have shown good antitumor activity.[64, 65,66]

Steroids have been reported tohave antibacterial properties[67] and they are very important compounds especially due to their relationship with compounds such as sex hormones.[68] Saponins which are known to produce inhibitory effect on inflammation.[69] Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness.[70,71]

All previous phytochemical investigations on Mediterranean species of *Ononis* genus has reaveled the presence of phenolics as aglycon flavonoids, some of them are rare.[72] Other study on alcoolic extract of *Ononis Angustissima* shows that *Ononis Angustissima* is very rich in flavonoids.[73,74] According to Boumaza *et al,* (2006), [75] all species of *Fabaceae* family including *Ononis Angustissima*, contain high levels of isoflavones, notably C-glycosylated isoflavones. Our present study confirms previous studies on the presence of flavonoids. As for other primary metabolites (glycosides) and secondary (tannins, steroids, terpenoids and saponins), no studies have addressed their presence or in the aqueous fraction or in alcoolic fraction. In our present research study, tannins, and terpenoids were strongly present in AEO,

while steroids, glycosides and saponins were present in traces. According to Datta *et al*, (1994).[76] the first formed assimilate in the plant will be the simple sugars which will be used for the plant metabolic activities.

Quantitative analysis

Total phenolics, flavonoid, tannin and β carotene determination. Because phenolics are of considerable interest as dietary supplements or food preservatives.[77] The total phenolic, flavonoid, tannin and β carotene contents in aqueous extract of *Ononis Angustissima* were analyzed and presented in **table 2**.

Table 2. Total phenolic, tannin flavonoid and β carotene contents of *Ononis Angustissima*.

Total phenolic mg GAE / g of dry extract	**Flavonoid** mg QuE / g of dry extract	**Tannin** mg TAE / g of dry extract	**β carotène** mg/ g of dry extract
118,55 ± 31,35	**8,05 ± 1,84**	**57,14 ± 9,55**	**0,009836 ± 0.0006**

Values are mean of triplicate determination (n = 3) ± standard deviation. GAE – Gallic acid equivalent. QuE – Quercetin equivalent. TAE – Tannic acid equivalent.

In the present study, the total phenolic and flavonoid contents were found to be 118,55 mg GAE and 8,05 mg QuE per g of dry extract, respectively, which were higher than methanolic fraction of *Ononis angustissima* (12,03 mg GAE and 2.35 mg Rutin Equivalent per g of dry weight, respectively) from *Sidi Makhlouf* (at 40 km at the north of *Laghouat*)-*Algeria*- .[20] The difference in content in favor of aquous extract can be explaned by using elevated temperature which has the effect to extract more amphiphilic compounds.[78]

On the other hand, acccording to the same study, [20] we note that the percentage of flavonoids compared to total polyphenols is not consistent with our present study. Effectively, according to Djeridane et al, (2010), [20] in the methanolic fraction, the flavonoids represents a rate of 18% compared to total phenols (2,35 to 12,03 mg, respectively). Our study shows a small percentage of flavonoids compared to total phenols which is around 6% (8,05 to 118,55 mg, respectively). This considerable difference suggests that the decoction (aqueous extract) contains less flavonoids, may be because alcohol solvents are more capable of increasing the permeability of cell walls and facilitating the extraction of a greater number of polar molecules of both medium and low polarity.[79] The study of Guettaf *et al,* (2016) [44]

on AEG confirms this difference. It shows a rate of flavonoids equal to 3 % compared to total phenols.

According to Bimark *et al,* (2010), [80] the higher concentrations of more bioactive flavonoid compounds were detected with higher polarity solvent as ethanol 70%.

As to other compounds such as tannins and β carotene, no previous studies have scrutinized their quantity in the plant. Our new study shows a significant content of tannins (57,14 mg TAE per g of dry weight) so a rate of 50 % compared to total phenols. By cons, β carotene presents traces.

On balance, *Genista Saharae* contains very active phenolic compounds such as tannins and flavonoids.

Antioxidant activity

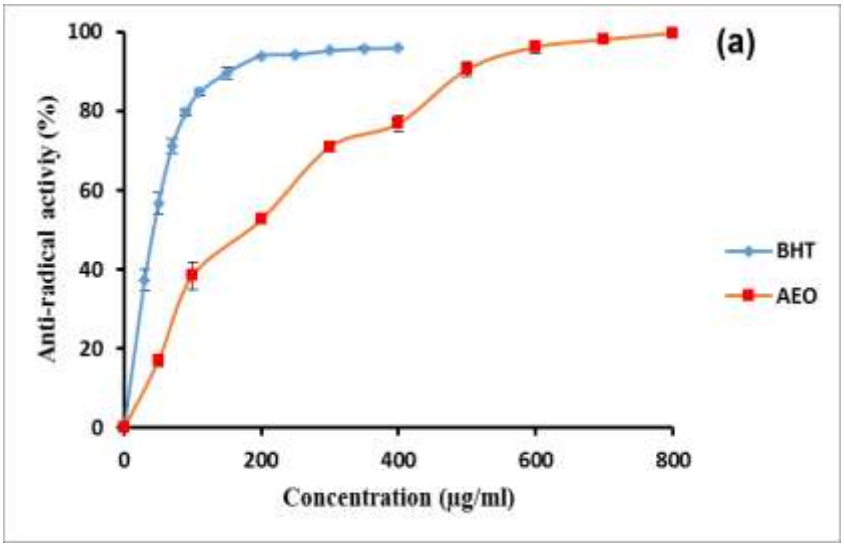
The antioxidant activities of polyphenols compounds may be attributed to various mechanisms such as prevention of chain initiation, binding with transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reducing potential and radical scavenging capacity.[81] Our present study scrutinizes the antioxidant activity by three methods; DPPH radical scavenging assay, reducing power assay and β carotene/linoleic acid bleaching assay.

DPPH radical scavenging assay. The free radical scavenging activity was measured by using The model of scavenging DPPH radical which is widely used for evaluating the free radical scavenging activities.[47] The results were expressed in percentage of inhibition of DPPH•. BHT was used as reference compound. **Fig. (3a**) presents the free radical scavenging ability of *Ononis Angustissima*. The results of the present study reavels that aqueous extract has a weak scavenging activity. However, the scavenging activities on DPPH• of AEO and BHT increased with the increase of concentrations in a dose dependent manner. At the concentration of 0.2 mg/ml, the DPPH scavenging activities of AEO and BHT were 53 and 94 %, respectively. Furthermore, the IC50 values of aqueous extract and BHT were found to be 191,35 ±14,80*** ($p < 0.05$) and 44,35 ±3,10 µg/ml, respectively **(Fig. 3b)**. We note a clear correlation between recorded antioxidant activity and total phenolic compounds and flavonoid contents (6% of total phénolics). However, EAO reveals an antioxidant activity relatively higher than that reported in an earlier study on aquoeus extract of *Génista Saharae*

(*Fabaceae*) (IC50=267,26 ± 8,43). The difference may be explained by the fact that EAO contains more flavonoids than EAG (8,05 and 3,60 mg QuE per g of dry extract, respectively).[44] Therefore, it is liable to correlate the antioxidant activity with the content of flavonoids.

On the other hand, antioxidant assessment results of our study registered a lower anti-radical property for aqueous extracts compared to previous study on alcholic extracts. Effectively, the evaluation of DPPH scavenging ability on ethanolic extract of *Ononis Angustissima* from *Tunisia*, shows a high scavenging activity with IC50 calculated as 24.48 ± 0.55 µg/ml.[82] This suggests that previous extract contain secondary metabolites with strong antioxidant activity.[47] It should be noted that the previous study has shown a high content of flavonoids contrary to ours (18%, and 6% of falvonoids to total phenolics, respectively).[44] The results are consistent with those of the literature considering the poverty of the aqueous extract in flavonoids in comparison to alcoholic extracts. Effectively, alchohol is more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells.[41] Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material.[83]

Thus, our present study was in accordance with previous reports. However the weakness of our extract is due either to the poverty of aqueous extract in term of flavonoids, either to the presence of other molecules which affect the ability of scavenging. [44]



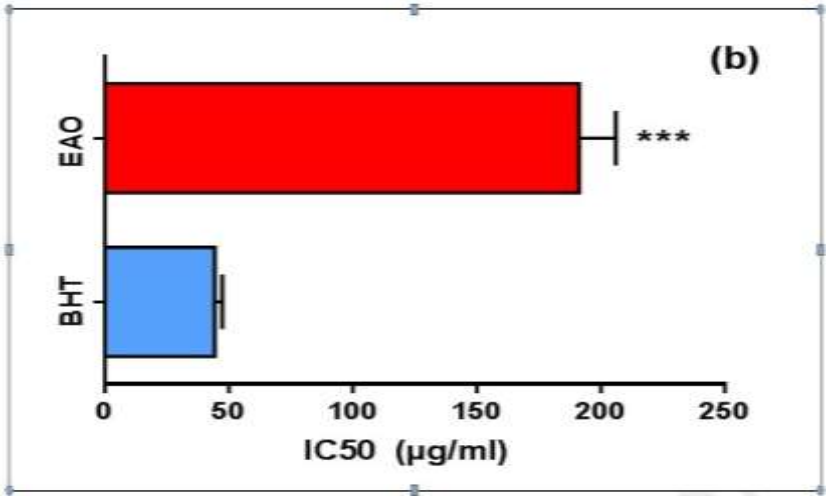


Fig 1. DPPH radical scavenging activity (a) and DPPH IC50 values (b) of *Ononis Aangustissima* and standard. Each value represents a mean ± SD (n=3), highly significant *(P≤0.05).**

Reducing power assay. In our present investigation, the transformation of ferric iron to ferrous iron was determined as reducing capacity. The reducing capacity of biomolecules as flavonoids may represent a significant indicator of its potential antioxidant ability. [77] As shown in **Fig. (2a)**, the reducing power of EAO and BHT increased with the increase of concentrations in a dose dependent manner. However, the reducing power of our extract was significantly lower than that of BHT ($p < 0.05$). At the concentration of 50 µg/ml, the reducing power of AEO and BHT were 0.034 and 1.8, respectively. Correspondingly, the IC50 values were 547*** and 15 µg/ml, respectively (**Fig.2b**). An earlier study on aqueous extract of *Génista Saharae* (*Fabaceae*) showed a relatively low reducing power compared to that of EAO, this allows to establish a correlation between the relatively high content in flavonoids of EAO and its reducing power (8,05 Vs 3,6 mg QuE per g of dry extract of EAO and EAG, respectively). However, this correlation does not apply to the content in polyphenols (130 Vs 118 mg GAE per g of dry extract of EAG and EAO, respectively). [44] On the other hand, another study on ethanolic extract of *Ononis angustissima*, revealed a very high reducing capacity whith an IC50 corresponding to 63.42 ± 0.78 µg/ml.[82] This large difference can be interpreted by the high percentage of flavonoids compared to total polyphenols in alcoolic extract compared with that of aquoeus extract (18% Versus 6% of flavonoids, respectively).[20]

On balance, the results obtained were expected given the poverty of aqueous extract in term of biomolecules responsibles for reducing power as flavonoids. Also, interferences due to

other compounds present in the extract for reducing power assay which is often a limiting factor.[44]

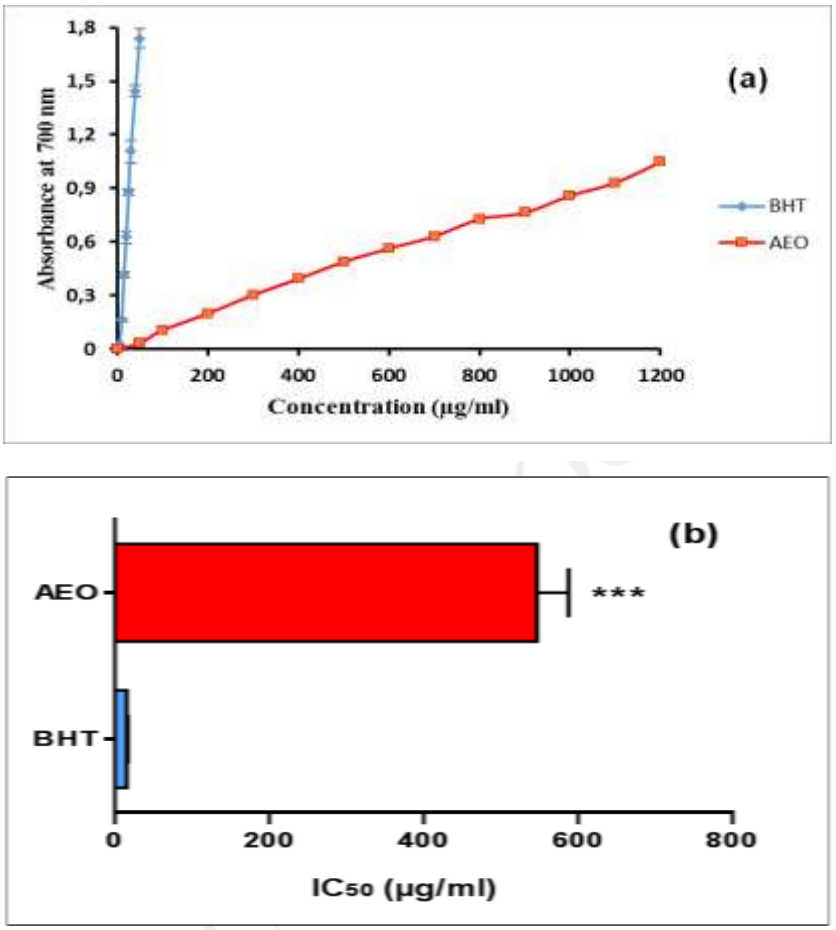


Fig 2. Reducing power (a) and IC50 values (b) of *Oninis Angustissima* and standard. Each value represents a mean ± SD (n=3), highly significant *(P≤0.05).**

β-carotene/linoleic acid bleaching assay. The test of β-carotene bleaching consists on inhibition of lipid peroxidation by donating a hydrogen atom. The occurence of biomolecules with antioxidant potential can prevent β-carotene bleaching by neutralizing the linoleate free radicals.[84] As shown in **Fig. (3a)**, there was a decrease in absorbance value due to the oxidation of β-carotene of all samples. At 2 mg/ml, AEO exhibited an interesting antioxidant potential estimated at 54 % as compared to standards BHA and BHT which were found to be 80 and 84 %, respectively. The results suggest that compounds and their redox properties may be responsible for the good antioxidant activity of EAO. This activity is due to a strong occurrence of phenolic compounds such as, phenols, saponins, terpenoids and specially flavonoids and tannins. In fact, flavonoids are accounted for its free radical as well as antioxidant activity.[1] Also, tannins may work as antioxidants to stop such damaging

reactions.[85] Contrary to flavonoids, tannins are considered superior antioxidants as their eventual oxidation may lead to enlargement of the number of reactive sites.[86] Our results were in concordance with that obtained by Guettaf *et al,* (2016) [44] who worked on a species of the same family *(Fabaceae)*, have shown that aqueous extract of *Génista Saharae* has a good antioxidant activity. The interesting results can be explained by the high occurrence of tannins ans flavonoids in aqueous fraction of *Ononis Angustissima*.[44]

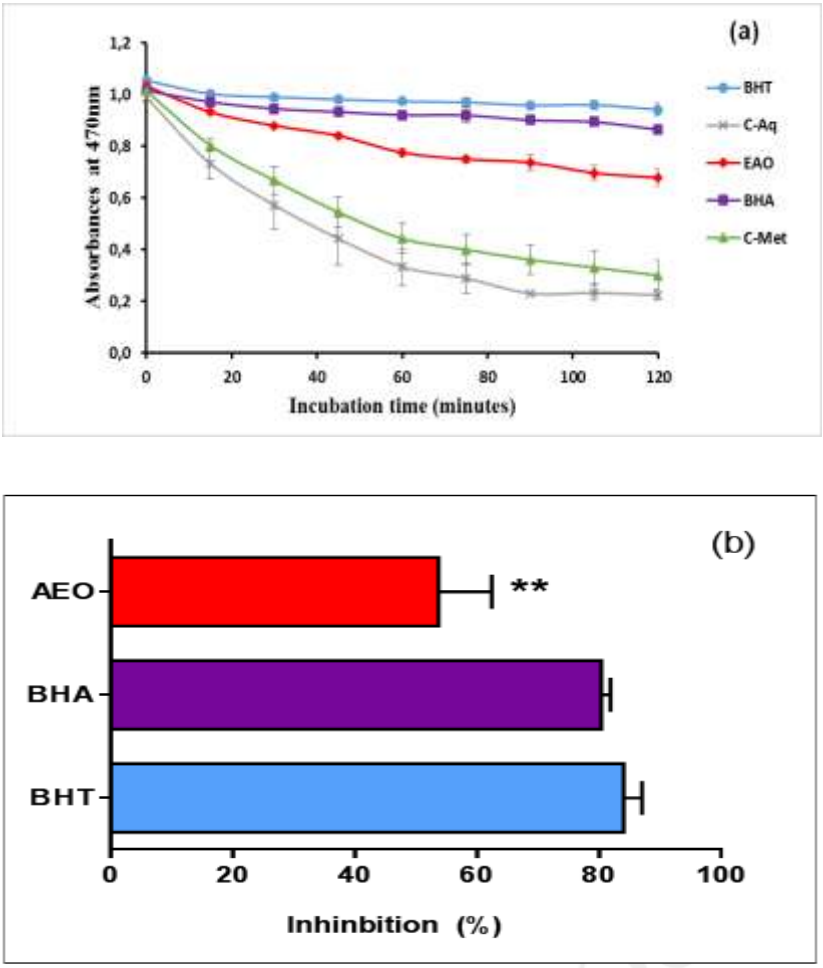


Fig 3. Effect of *Ononis Angustissima* and standards on oxidation of β-carotene/linoleic acid (a) and inhibition pourcentage β-carotene bleaching (b). Each value represents a mean ± SD (n=3), significant **(P≤0.01).

CONCLUSION

The phytochemical screening of this investigation attested the presence of several metabolites in aqueous extract of *Ononis Angustissima* such as, flavonoids, tannins, terpenoids, steriods, saponins and glycosides. In addition, the quantitative analysis shows a good occurence of total phénolics flavonoids and particullary tannins. Also, aerial parts were found to possess an

interesting antioxidant capacity by protecting β carotene from auto-oxidation which justifies its use by traditional healers. Inhibition pourcentages of AEO was found to be close to that of referencial antioxidants (54%). The presence of tannins and flavonoids particulary, and other biomolecules as terpenoids and saponins, may be responsible for different redox properties. Thus, *Ononis Angustissima* could be candidate for a good source of antioxidant compounds. Further studies are currently underway to assess other biological activities and to identify the active component responsible for thier antioxidant property.

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