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Phytochemical screening and antioxidant activity of aqueous extract of *Genista Saharae* (*Coss.* & *Dur.*)

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ABSTRACT

The need for antioxidant agents, which can prevent oxidative stress, has become a major priority. Saharan plants could constitute a reservoir of effective biomolecules useful as antioxidants. Genista saharae, wich is anendemic plant of Algeria,is a leafless spontaneous fabaceae called "Tellegit" by local people. As it is known to be a source of chemical compounds which are characterized by antioxidant properties, this medicinal shrub is used in traditional pharmacopoeia. The originality of the present study consists in scrutinizing the phytochemical composition and evaluating the antioxidant activity of aerial parts of aqueous extract of Genista Saharae (AEG) in conditions corresponding to its traditional use. Phytochemical screening was performed to assess the qualitative chemical composition of (AEG) using precipitation and coloration reactions. In addition, the total phenolics, flavonoids, tannins and β carotene contents were determined by using spectrophotometric methods. Finally, AEG was assayed to determine its antioxidant activities using 3 methods: DPPH, reducing power and β carotene bleaching tests. The results reveal the presence of several biomolecules in AEG such as phénolics compounds, flavonoids, alkaloids, tannins, terpenoids, glycosides, steroids and saponins. Besides, the quantitative analysis show a considerable total phenolics, tannins and β carotene contents. In sum, it was found that EAG possess a high antioxidant activity against bleaching of β carotene, which justifies its use by traditional healers.Consequently, Genista Saharae is a good source of antioxidants. Its good antioxidant activity may be explained by the occurence of tannins or other phenolic compounds such as terpenoids, β carotene and saponins. .

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

INTRODUCTION

Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant system [1]. Free radicals are widely believed to be involved in the etiology of many diseases such as ageing, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation as indicated by the signs of oxidative stress [2]. This brings the need for antioxidant agents wich can prevent oxidative stress [1] by retarding or inhibiting the oxidation possibly by free radicals in biological system [3].However, synthetic antoxidants such as butylated hydroxyanisole and butylated hydroxytoluene are considered to be responsible for liver damage and carcinogenesis [4]. Therefore, it is essential to develop natural non-toxic antioxidants to protect human body from free radicals and retard the progress of many chronic diseases [5]. Several plants are known to be a source of chemical compounds wich are characterized by antioxidant properties. These antioxidants compounds could possess the ability to protect the cellular organelles from damage caused by free radicals induced oxidative stress [6]. Saharan plants are known by their resistance to several stress factors. Under extreme climatic conditions, Saharan plants could constitute a reservoir of new natural, safe and effective biomolecules potentially useful as antioxidants [7]. The genus *Genista* is present in Algeria with 25 species and sub-species from which 11 are

endemic [8]. Flavonoids, isoflavonoids and alkaloids are known to be present in *Genista* species [9, 10]. *Genista saharae* is an endemic Saharan medicinal shrub used in traditional pharmacopoeia by local population against various diseases as an herbal remedy for inffections of the respiratory system [11] . It is also used for feeding animals. Effectevely, it is frequently appreciated by camels because of its serious urine retention capacity in this livestock [12, 13]. In addition to their pastoral qualities, it is suitable for sand stabilization and combating desertification[14].

Some previous studies have evaluated the in chemical composition and antioxidant activities of alcoholic extracts of this genus[15, 11, 16, 17]. Unfortunately, to date, there are still no phytochemical and biological data available about this endemic Algerian shrub. Also, there is no literature report about the antioxidant activity for its aqueous extract. Therefore, in the present paper, we scrutinize the phytochemical composition and evaluate the antioxidant activity of aeriel parts of *Genista Saharae* in conditions corresponding to its traditional use.

MATERIALS AND METHODS

Plant material. The aerial parts of the plant were harvested in *Maiter Oued* in the region of *Bou Saada*, south of *M'sila*,located at a longitude of 4° 10’ Est and latitude of 35°12’ North at an altitude of 905 meters above the level of the sea.The plant was collected during flowering stage in March 2015 and the biological tests were conducted one month later. Their identification was carried out by Pr.*RAMDHANI Messaoud* 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 [18] 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 andcentrifuged 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 [19].**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 *Génista Saharae*(AEG)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 [20, 21].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 [22].

Screening for flavonoids. For the confirmation of flavonoid in the selected plant, 0.5 g of AEG 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[23].

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 mixturewas heated, then filtered and afew drops of picric acid in a mixture was added. Formation of yellow color precipitate indicates the presence of alkaloids [23].

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 [24].

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 [25].

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 [26].

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[27].

Screening for saponins (Bubble test). 5 mlof aqueous extract was shaken vigorously for 2 minutes. The appearance of foam that persisted for at least 15 min confirmed the presence of saponins [28].

Quantitative analysis

Total phenolics determination. Total phenolic content was estimated by the Folin–Ciocalteu method [29]. 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 *Genista Saharae*, 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 [30]. 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 *Genista Saharae*, and calculated as mean value ± SD (n = 3).

Tannin determination. Tannins content was evaluated using the hemoglobin precipitation assay as described by [31]. 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 *Genista Saharae*, and calculated as mean value ± SD (n = 3).

B carotene determination. The content of β carotene was determined by the spectrophotometric method of [32]. Briefly, 100 mg of aqueous extract of *Génista Saharae* 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 *Génista Saharae* (AEG) was measured by DPPH•using the method of [33].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 EAG and 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 EAG 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 [34]. 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 mltrichloroacetic 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[35].

β-carotene/linoleic acid bleaching assay. The ability of AEG to prevent the bleaching of ß carotene was determined as described by [36]. 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 aqueousdecoctionof aerial parts of *Genista Saharae* was found to be in order of 10.33 % in term of dry weight.Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Generally, the choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay[37]. Therefore, we have chosen water as solvent in our present study.Water is universal solvent, used to extract plant products by traditional healers. In addition, water soluble phenolics have important significance as antioxidant compound[38].A previous work shows that the yields of the alcoholic extracts (MeOH) of different aerial parts of *Genista Saharae*were ranged from 10.93 to 18 %[15], which is more or less higher than that of aqueous extraction (10.33%). This result is expected 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 [39]. However, the aqueous extraction is carried out by high temperature decoction for 20 min. In fact, [40]reported that aqueous extraction efficiency increases with temperature. This is explained by the fact that the high-temperature water causes disruption of cells which promote penetration and solubilization of the solvent molecules [41]. Heat may, however, lead to the degradation of heat labile molecules [39]. This is why, the decoction was carried out for a shorter time.

Phytochemical analysis

Qualitative analysis. Qualitative phytochemical screening of aqueous extract from *Genista Saharae* is summarized in the **table 1**. The sample showed the enormous occurrence of phytochemicals considered as active medecinal chemical constituents [23].

Table1. Qualitative analysis from *Genista Saharae (Fabaceae)*

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

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

Phenols, flavonoids, tannins, alkaloids, terpenoids and glycosides are strongly present in aqueous extract, while steroids andsaponins show traces.These bioactive components are naturally occurring in most *Genista* species and known to possess interesting biological activities [42, 43].Several studies have shown that a diet rich in fruit and vegetables has an important role in reducing the incidence of diseases. Some of these preventive actions have been related to the presence of bioactive substances such as polyphenols[44, 45].Flavonoids are characterized by a common benzopyrene ring structure. The biological functions of flavonoids, apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors. Flavonoids reduced cancers by interfering with the enzymes that produce estrogen [46]. Also, epidemiologic studies recommend that coronary heart disease is opposed by dietary flavonoids[23].Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, hemorrhoids and diarrhea [47].It hasalso been reported to posses wound healing properties through anti-inflammatory, analgesic [48]and antioxidantmodalities [49].Plants having alkaloids are used in medicines for reducing headache and fever. These are attributed with antibacterial and analgesic properties [44]. Terpenoids are reported to have anti-inflammatory, anti-viral,anti-malarial, inhibition of cholesterol synthesis and anti-bacterial properties [50].Glycosides are nonvolatile and lack fragrance and serve as defense mechanisms against predation by many microorganisms, insects and herbivores [51].Steroidsare known to be important for their cardiotonic activities ; they possess insecticidal and anti-microbial properties. They are routinely used in medicine because of their profound biological activities [47].Saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells, particularly lung and blood cancers. It served as natural antibiotics, which help the body to fight infections and microbial invasion [52].All previous phytochemical investigations on *Genista*species has reaveled the presence of phenolics as flavonoids, isoflavonoids and alkaloids[53, 54, 55, 56, 57].Other studies on methanolic, ethyl-acetate and ethanolic extracts of *Genista saharae* show the presence of flavonoids [15,11], alkaloids and isoflavones[58,16].According to these authors, *G.saharae*, like the other species of Fabaceae family, contains high levels of isoflavones, notably C-glycosylated isoflavones. This compound (C-glycosylated isoflavones) qualified a new identifiable isoflavone [59]. Other reports also indicated that *Genista* species contain a variety of secondary metabolites of various types, especially isoflavonoids, which have beenshown to be biologically active [60, 61].Our present study confirms previous studies on the presence of flavonoids and alkaloids. 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 another fraction. In our recent research studies, tannins, glycosides and terpenoidss were strongly present in AEG, while steroids and saponins were present in traces. The presence of glycosides is justified because the first formed assimilate in the plant will be the simple sugars which will be used for the plant metabolic activities. The excesswill be stored in their reserve organs[62]. Increased total sugars in tubers may be attributed to the high partitioning efficiency and increased efficiency of the sink to accumulate assimilates in the tubers [47].

Quantitative analysis

Total phenolics, flavonoid, tannin and β carotenedetermination. Recently, there has been increasing interest in discovering natural antioxidants, especially those of plant origin. Natural antioxidants derived from plants, chiefly phenolics, are of considerable interest as dietary supplements or food preservatives[5].Hence, an attempt was made to quantify some secondary metabilites of aqueous extract of *Genista saharae*.The total phenolic, flavonoid, tannin and β carotene contents were analyzed and presented in **table 1**.

Table 2. Total phenolic, tannin flavonoid and β carotene contents of *Genista Saharae*

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
130,44 ± 15,43	**3,60 ± 0,72**	**12,76 ± 2,07**	**0,02636±0.003**

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 130,44 mg GAE and 3.61 mg QuE per g of dry extract, respectively, which were lower than ethyl acetate fraction (EAF) of *Genista saharae* (425,28 mg GAE and 242 mg QuE per g of dry weight, respectively) from Ghardaya-Algeria-[11]. The difference in content in favor of organicextract (ester of ethanol and acetic acid) is logical, because the water is not a good solvent for the phenolic compounds contrary to alchoholic solvents. Indeed, the aqueous extraction, decoction (boil for 20 minutes) corresponds to the traditional use of the plant. However, water is not the ideal solvent for a number of bioactive components in plants. It allows to preferentially extract polar compounds, but at elevated temperature, it can also extract some amphiphilic compounds [63]. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol [64].Whereas, alchohol is more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells [65]. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material [66].Moreover, the course of this extraction by alchoholic solvent at room temperature as well as the exhaustion of the solvent at reduced pressure provides the maximum of compounds and prevents their denaturation or likely modification due to the high temperatures used in other extraction methods.

On the other hand, acccording to previous studies, we note that the percentage of flavonoids compared to total polyphenols are not consistent with our present study. Effectively, according to [11], in the ethyl acetate fraction, the flavonoids represents a rate of 53% compared to total phenols (245 to 459,28 mg, respectively). Our study shows a very small percentage of flavonoids compared to total phenols which is around 3% (3,61 to 130 mg, respectively).This large difference suggests that the decoction(aqueous extract) contains less flavonoids. Another study on methanol extract confirms this difference, it is that of [15].This study shows a rate of flavonoids equal to 28% compared to total phenols (2,61% to 9,33%, respectively). It was reported that the higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased [67].As to other compounds such as tannins and β carotene, no studies have scrutinized their quantity in the plant. Our new study shows a significant content of tannins and Beta carotene (12,76 mg TAE per g and 0,026 mg per g of dry weight, respectively).

On balance, *Genista Saharae* contains very active phenolic compounds such as tannins, beta carotene andin particular flavonoids and isoflavones [16], notably C-glycosylated isoflavones [59].These compounds which is accounted for its free radical as well as antioxidant activity [68]. It was also reported that the oil of *Genista Saharae* very rich in fatty acids [17].

Antioxidant activity

Most plant-derived polyphenols exhibit strong antioxidant potentials. The antioxidant activities of these compounds have been attributed to various mechanisms which is established by various assay procedures [69]., such as prevention of chain initiation, binding with transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reducing capacity and radical scavenging ability [70]. Our present study tests 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 DPPH assay. The model of scavenging DPPH radical has been widely used for estimating the free radical-scavenging activities of plant extracts/antioxidants [1]. DPPH• radical is a stable free radical, so the antioxidants are able to reduce DPPH• radical (purple) to the non-radical form DPPH-H (yellow). The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities [5].The results were expressed in percentage of inhibition of DPPH•. BHT was used as reference compound. **Figure (3a**) shows the free radical scavenging ability of *Genista Saharae*. The results of the present investigation reavels that aqueous extract has a weak hydrogen donating ability. As shown, the scavenging activities on DPPH• of AEG and BHT increased with the increase of concentrations in a dose dependent manner. At the concentration of 0.2 mg/ ml, the DPPH scavenging activity for AEG and BHT was 42 % and 94 %, respectively. Furthermore, the IC50 value of aqueous extract and BHT were found to be 267,26 ± 8,43*** ($p < 0.05$) and 44,35 ±3,10 µg/ml, respectively**Fig.(3b)**. We notea clear correlation between recorded antioxidant activity and total phenolic compounds and flavonoid contents (3% of total phénolics).On the other hand,

antioxidant assessment results of our study registered a lower anti-radical property for aqueous extracts compared to previeous studies. Effectively, a combination of a DPPH scavenging assay with HPTLC-MS on methanolic extract, shows a high scavenging activity with IC50 calculated as 8.27 µg/ml [15]. Furthermore, another evaluation of antioxidant capacity of Ethyl acetate fraction(EAF), has also provided interesting results. The DPPH test revealed that the EAF of *G. saharae* possessed a good antioxidant capacity of 26.52 mg VCE/g plant extract [11]. This suggests that previous extracts contain secondary metabolites with strong antioxidant activity[1].It should be noted that two previous studies have shown a high content of flavonoids contrary to ours (28%, 57% and 3% of falvonoids to total phenolics, respectively). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports [71, 72]. Specially, flavonoids are active in the process of scavenging of free radicals[73]. According to [15], in *Génista Saharae* both polar and apolar isoflavones were found to be the main contributors to antioxidant activity. Whereas, other phenolics did not react with DPPH. Thus, our present study was in accordance with previous reports. However the weakness of our extract is due either to the poverty of our aqueous extract in term of flavonoids, either to the presence of other molecules which affect the ability of scavenging in terms of hydrogen donating ability .

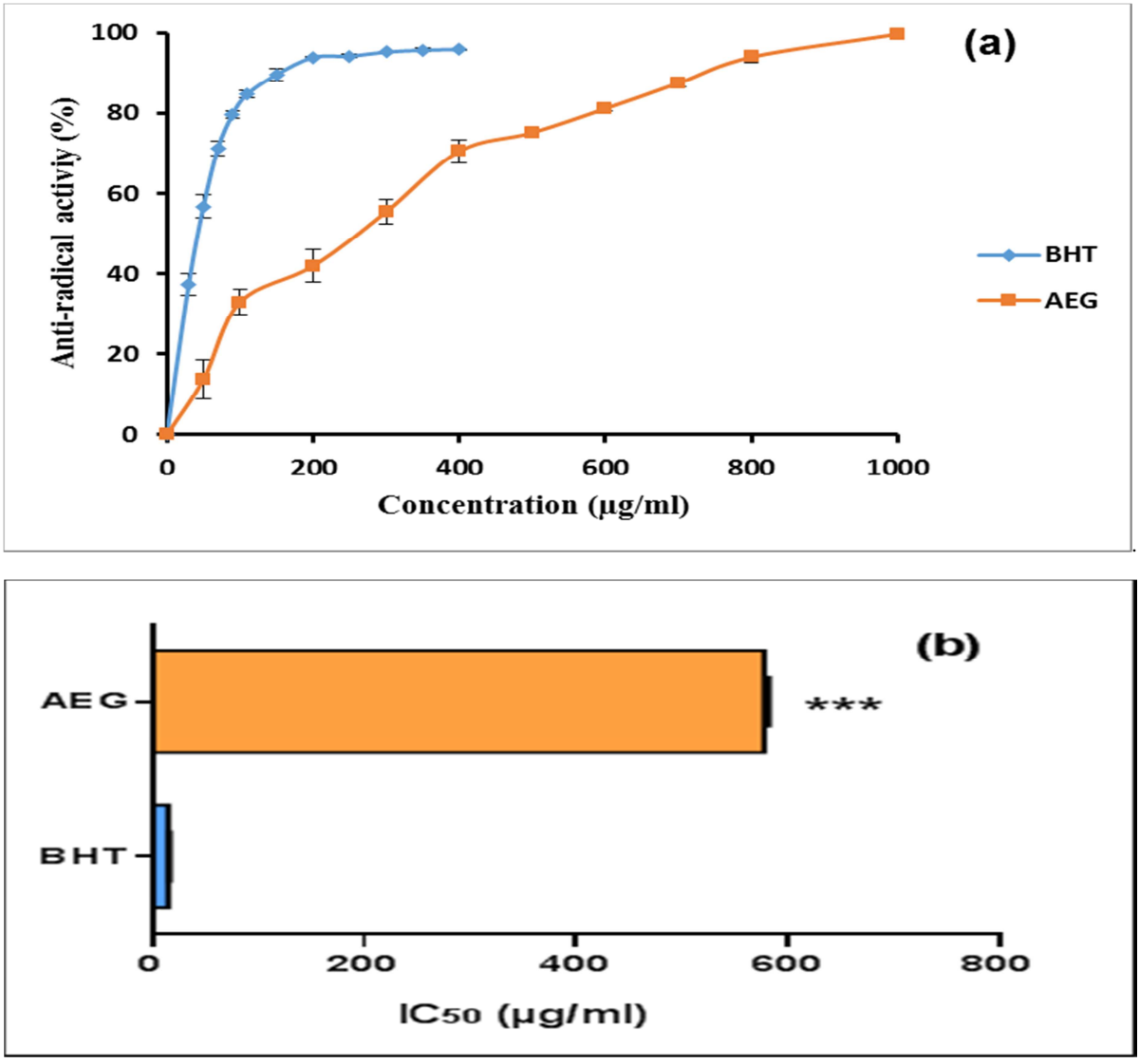

Figure 1. DPPH radical scavenging activity (a) and DPPH IC50 values (b) of *Genista Saharae* and standard
*Each value represents a mean ± SD (n=3), highly sig nificant ***(P≤0.05)*

Reducing power assay. The antioxidant activities of natural components may have a reciprocal correlation with their reducing capacity [74, 75].Thus the reducing capacity of these compounds may serve as a significant indicator of its potential antioxidant activity [5]. High reducing power of flavonoids suggested their remarkable potency to donate electrons to reactive free radicals, thus converting them into morestable non-reactive species and finally terminate the free radical chain reaction [76]. In our present study, the transformation of Fe3+ to Fe2+ was determined as reducing capacity. As shown in **Fig. (2 a)**, the reducing power of aqueous extract 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 AEG and BHT were 0.03 and 1.8, respectively. Accordingly, the IC50 values were 578*** and 15 µg/ml, respectively (**Fig.2b**). Considering the poverty of aqueous extract in term of compounds responsibles for reducing power(flavonoids), the results obtained were justified. Adding to that statement, interferences due to other compounds present in the extract for reducing power assay which is often a limiting factor. These results were consistent with previous tests concerning flavonoid amountand radical scavenging activity.

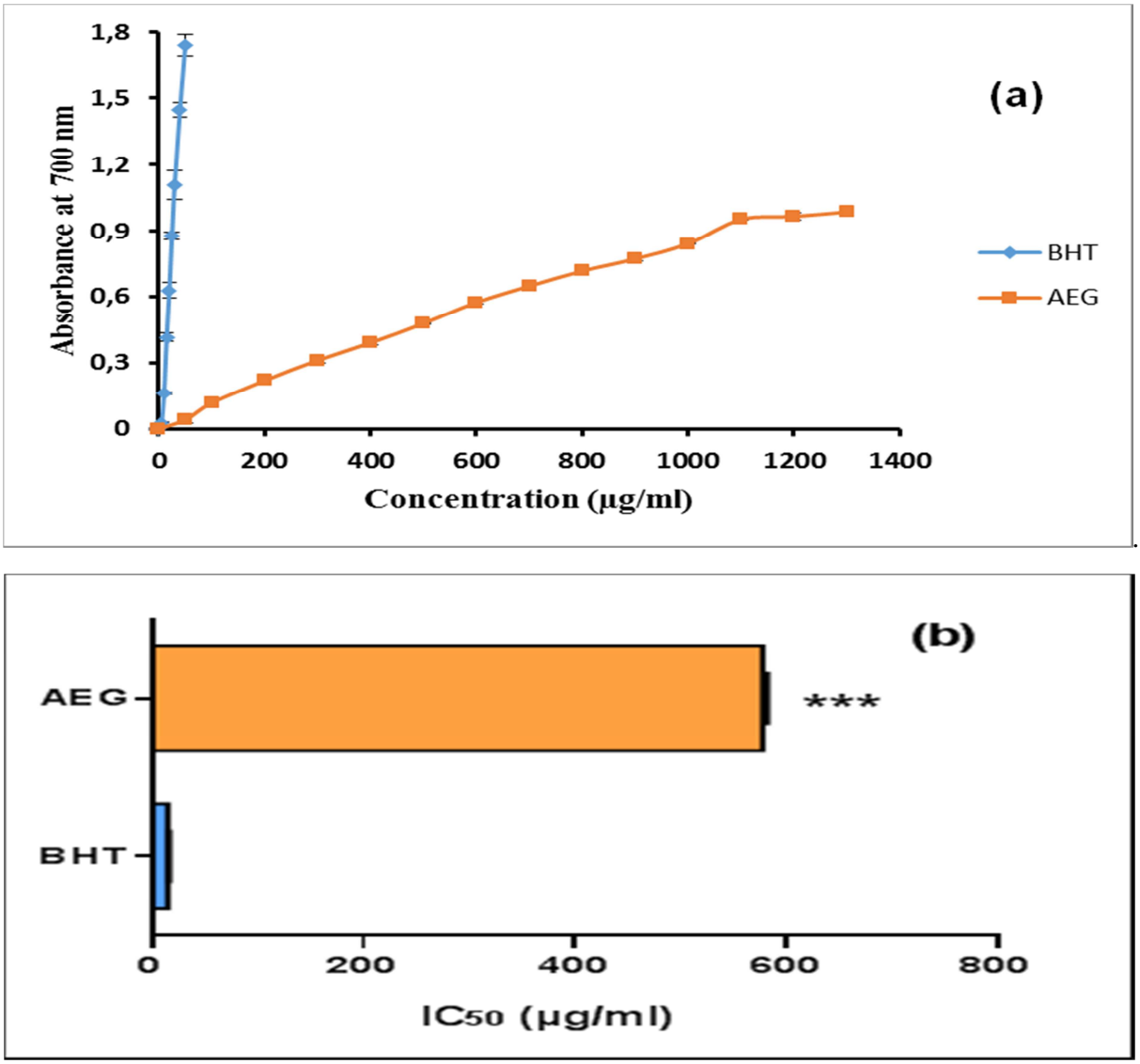

Figure 2. Reducing power (a) and IC50 values (b) of *Genista Saharae* and standard
*Each value represents a mean ± SD (n=3), highly significant ***(P≤0.05)*

β-carotene/linoleic acid bleaching assay. β-carotene bleaching test is based on inhibition of lipid peroxidation by donating a hydrogen atom. The oxidation of linoleic acid produces hydroperoxides derived free radicals which attack the chromophore of β-carotene resulting in bleaching of the reaction emulsion [77].The presence of antioxidant compounds in extract can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radicals and other free radicals formed in the system [78].**Fig. (3a)** showed that there was a decrease in absorbance value due to the oxidation of β-carotene of all samples. At 2 mg/ml aqueous extract exhibited a high antioxidant activity estimated at 77 % as compared to standards substances BHA and BHT which were found to be 80% and 84 %, respectively, by protecting β-carotene from auto-oxidation, due to free radical chain generation from linoleic acid peroxidation [43].The difference of inhibition between AEG and both of standards was statistically non significant (ns ; p>0,05). Indeed, The abilities both extract and standard to retard the lipid peroxidation were in order of BHT (84 %) > BHA (80 %)> EAG (77 %) (**Fig. 3b**). The results suggest that compounds and their redox properties may be responsible for the antioxidant activity of EAG. This activity is due to a strong occurrence of phenolic

compounds such as β carotène, saponins, terpenoids, phenols and tannins[68].In fact, tannins may work as antioxidants to stop such damaging reactions[79].Tannins are considered superior antioxidants as their eventual oxidation may lead to oligomerization via phenolic coupling and enlargement of the number of reactive sites, a reaction which has never been observed with the flavonoids themselves [69]. The obtained results were in accordance with our phytochemical screening.

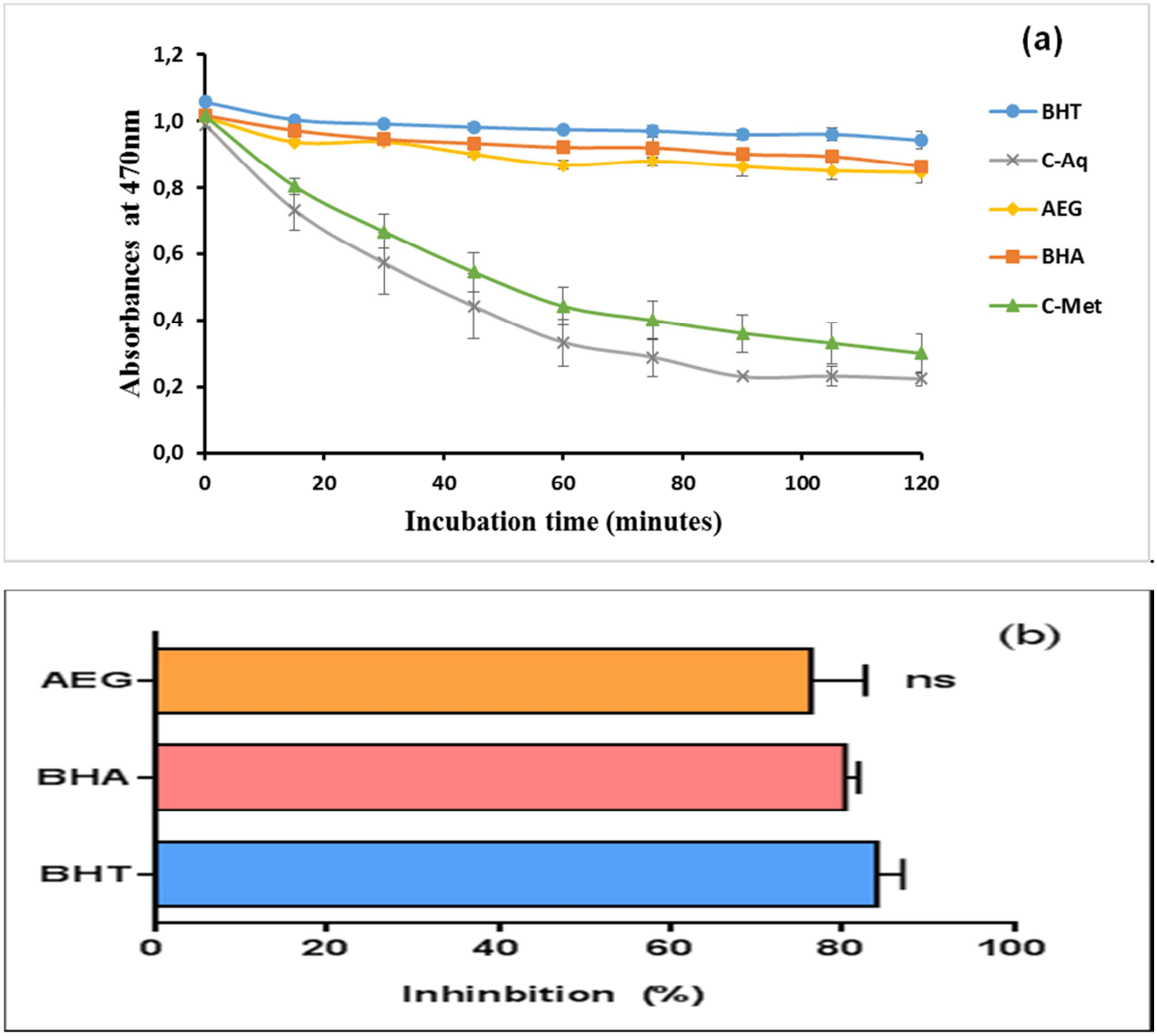

Figure 3. Effect of *Genista Saharae* and standards on oxidation of β-carotene/linoleic acid (a) and inhibition pourcentage β-carotene bleaching (b)

Each value represents a mean ±SD (n=3), non significant (P>0.05).

CONCLUSION

The aim of our present study is to scrutinize the qualitative and quantitative constitution and antioxidant proprieties of endemic sahran *Genista Saharae*. Prelimenary screening phytochemical reveals the presence of various compounds such as flavonoids, tannins, terpenoids, alkaloids, steriods, saponins and glycosides. Furthermore, the quantitative analysis show a considerable total phénolics and tannins contents. On the other hand, aerial parts possesses a high antioxidant capacity by protecting β carotene from auto-oxidation which sustains its use by traditional healers.Inhibition pourcentages of AEG, BHA and BHT were found to be 77%, 80%and 84%, respectively, which means a statistically non sgnificant difference (P >0.05). The presence of tannins particulary, and other bioactive components as terpenoids, β carotene and saponins, may reflects the antioxidants properties. This work is the first report which correlates the presence of tannins with the high antioxidant activity in term of inhibition of peroxidation of lipids. Further studies should be carried out to isolate and identify active compounds and to understand the mechanism of action against the observed antioxidant system.

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