Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

PHYTOCHEMICAL STUDY AND EVALUATION OF ANTIOXIDANT ACTIVITY OF *SOPHORA JAPONICA* L. (FABACEAE) OF THE REGION OF BOUMERDES (ALGERIA)

BENHABYLES-BOUTTABA Narimen[1*], BOUCHENAK Ouahiba[2], BOUMAZA Sarah[1], LAOUFI Razika[3], YAHIAOUI Karima[1], SADAOUI Nesrine[2], TOUBAL Souheyla[1] and ARAB Karim[1]

1. Laboratory of Valorization and Conservation of Biological Resources (VALCOR) -Faculty of Science -University M'Hamed Bougara – Algeria.
2. Bioinformatics, Applied Microbiology and Biomolecules. Faculty of Science, University of M'hamed Bougara Boumerdes Algeria.
3. Laboratory of Technologies Soft and Valorization of Biological Materials and BiodiversityFaculty of Science University M'hamed Bougara – Algeria.

Reçu le 05/12/2020, Révisé le 15/05/2021, Accepté le 18/05/2021

Summary

Description of the subject : Natural substances derived from plant biomass have multiple interests that are used in biotechnology as in the food, cosmetic and pharmaceutical industries. Among these compounds we find a large part of the secondary metabolites which have especially been illustrated in therapy. Traditional herbal remedies have long been used without knowing what their actions were due to.

Objectives : Studies of secondary metabolites are the subject of much research based on in vitro cultures. This is particularly the case for the phenolic compounds which are the subject of our study, compounds widely used in therapy as vasculoprotectors, anti-inflammatories, enzyme inhibitors, antioxidants and anti-radicals. In this context, the present work concerns a phytochemical and antioxidant study from the plant Sophora japonica traditionally used to treat several diseases.

Methods : We focused our work on the extraction of polyphenols by the maceration method, as well as their identification. Quantification by spectrophotometric methods allowed us to determine the contents of total phenols by the Folin-Ciocalteu reagent, flavonoids by Aluminum trichloride, tannins condensed by vanillin, anthocyanins by the differential PH method, and as carotenoids by the mixture of solvents (hexane/acetone/ethanol). Research of the effect of the phenolic extract on the antioxidant power was carried out by five methods : the method of reduction of the free radical DPPH, the method of reduction of ferrous iron (FRAP), the bleaching test of β- carotene / linoleic acid, the ABTS radical cation reduction test and total antioxidant capacity.

Results : The extraction of polyphenols yielded a métanolique extract with a good yield of about 66.5%. The determination of total polyphenols, flavonoids, condensed tannins, anthocyanins, carotenoids and the methanolic extract revealed respective contents of 0.011mg/g, 15,65mg/g, 0.038 mg/g, 0,085mg/g, 1.333mg/g of dry dry plant material. For the DPPH test, the results reveal that the methanolic extract has an anti-radical activity greater than that of the standards (BHT and ascorbic acid) ($p \leq 0.01$). The iron reduction technique FRAP revealed a higher reducing capacity of the methanolic extract compared to quercetin and rutin ($p \leq 0.01$). The β-carotene bleaching test also showed that quercetin is the most active as inhibitors of the oxidation of linoleic acid compared to the methanolic extract, rutin and BHT. For the ABTS ° + test, the results reveal that the methanolic extract of sophora pods is more active as an inhibitor compared to BHT. The total antioxidant capacity test also showed that the methanolic extract expresses strong antioxidant power.

Conclusion : The results of this work have enabled us to affirm that the extract of the plant studied has very good antioxidant properties which could allow us to recommend them in biotechnology.

Keywords: Oxidative stress, antioxidant, polyphenol, DPPH, FRAP, ABTS, β-carotene, CAT.

ETUDE PHYTOCHIMIQUE ET EVALUATION DE L'ACTIVITE ANTIOXYDANTE DE *SOPHORA JAPONICA* L. (FABACEAE) DE LA REGION DE BOUMERDES (ALGERIE).

Résumé

Description du sujet: Les substances naturelles issues de la biomasse des végétaux ont des intérêts multiples mis à profit dans la biotechnologie tant dans l'industrie alimentaire, cosmétique que pharmaceutique. Parmi ces composés on retrouve en grande partie les métabolites secondaires qui se sont surtout illustrés en thérapie. Les remèdes traditionnels à base de plantes ont longtemps été utilisés sans savoir à quoi leurs actions étaient dues.

Objectifs: Les études des métabolites secondaires font l'objet de nombreuses recherches, c'est le cas notamment des composés phénoliques, largement utilisés en thérapeutique comme vasculoprotecteurs, anti-inflammatoires, inhibiteurs d'enzymes, antioxydants et anti-radicalaires. Dans ce contexte, le présent travail porte sur une étude phytochimique et antioxydante de la plante *Sophora japonica* L. utilisée traditionnellement pour traiter plusieurs maladies.

Méthodes: Nous avons concentré notre travail sur l'extraction des polyphénols par la méthode de macération, ainsi que leur identification. La quantification par des méthodes spectrophotométriques nous a permis de déterminer les teneurs en phénols totaux par le réactif de Folin-Ciocalteu, en flavonoïdes par le trichlorure d'aluminium, en tanins condensés par la vanilline, en anthocyanes par la méthode du PH différentiel, et en caroténoïdes par le mélange de solvants (hexane/acétone/ éthanol). La recherche de l'effet de l'extrait phénolique de *S. japonica* sur le pouvoir antioxydant est réalisée par cinq méthodes: la méthode de réduction du radical libre DPPH•, la méthode de réduction du fer ferreux (FRAP), le test de blanchissement du β-carotène/acide linoléique, le test de réduction des cations radicalaires ABTS et la capacité antioxydante totale.

Résultats: L'extraction des polyphénols a donné un extrait métanolique avec un bon rendement, il est de l'ordre de 66,5%. Le dosage des polyphénols totaux, des flavonoïdes, des tanins condensés, des anthocyanes et des caroténoïdes de l'extrait méthanolique a révélé des teneurs respectives de 0,011mg/g, 15,65mg/g, 0,038 mg/g, 0,085mg/g, 1,333mg/g de matière végétale sèche. Pour le test de DPPH, les résultats révèlent que l'extrait méthanolique a une activité anti-radicalaire supérieure à celle des standards (BHT et acide ascorbique) ($P \leq 0,01$). La technique de réduction du fer FRAP a révélé une capacité réductrice plus élevée de l'extrait méthanolique par rapport à la quercétine et la rutine ($P \leq 0,01$). Le test de blanchissement du β-carotène a également montré que la quercétine est la plus active en tant qu'inhibiteur de l'oxydation de l'acide linoléique par rapport à l'extrait méthanolique, la rutine et le BHT. Pour le test ABTS°+, les résultats révèlent que l'extrait méthanolique des gousses de sophora est plus actif comme inhibiteur par rapport au BHT. Le test de la capacité antioxydante totale a également montré que l'extrait méthanolique exprime un fort pouvoir antioxydant.

Conclusion: Les résultats de ce travail nous ont permis d'affirmer que l'extrait de la plante étudiée possède de très bonnes propriétés antioxydantes qui pourraient nous permettre de les recommander en biotechnologie.

Mots clés: Stress oxydatif, antioxidants, polyphenols, DPPH, FRAP, ABTS, β-carotène, CAT.

* Corresponding author : BENHABYLES-BOUTTABA Narimen, E-mail : n.benhabyle@univ-boumerdes.dz

INTRODUCTION

Medicinal plants have been used since ancient times to relieve and cure human diseases. Indeed, their therapeutic properties are due to the presence of natural bioactive compounds called secondary metabolites [1]. Currently, the development of the toxicity of synthetic antioxidants has led researchers to tap into the plant world in search of effective natural molecules devoid of any adverse effect. [2]. In addition, in recent years, herbal medicines have become increasingly popular in certain regions of the world. [3]. For plants whose chemical composition is not known, which is the case for several medicinal species, and to get an idea of the activity of the plant, we must seek to identify certain active ingredients. Among the major families of secondary metabolites of plants which are endowed with pharmacological activity are the alkaloids, the saponosides, the essential oils and the polyphenols [4]. They possess a wide range of biological activities related to their simplistic nature and their affinity for proteins and metal ions. These metabolites have well-established antioxidant properties and are linked to the inhibition of oxidation both in the food sector (lipid oxidation) and physiological (oxidative stress). These substances are attracting much interest in several fields, in particular in the food industry by their implications on the flavor of food and their impact on the preservation of food products [5]. The oxidation phenomenon generated by free radicals affects both the human body and the various existing food groups [6]. Under certain conditions, there appears an imbalance in the oxidant / antioxidant balance caused by an exaggeration of free radicals or by a decrease in antioxidant defenses. This damage is achieved by the attack of free radicals on various biomolecules, in particular proteins, lipids and DNA, ultimately resulting in the degradation and death of cells [7]. The north of Algeria has an extremely rich and varied flora, characterized by its originality on a systematic level (numerous endemic plants) and its wide use in folk medicine. These characteristics make the study of flora of great scientific interest in the phytochemical field [8]. Among the families of medicinal plants that make up the plant cover is the Fabaceae family. *Sophora japonica* L. is the pagoda tree of the fabaceae family, native to East Asia. This tree is a deciduous legume mainly present in china which was introduced in Japan.

The present work is interested in the demonstration of the characteristics of this plant and the evaluation of the antioxidant activity of its polyphenolic compounds.

MATERIAL AND METHODS

1. Plant material

The plant material consists of the pods of Sophora japonica L. harvested at the beginning of March 2018. This collection was made in the central region of Boumerdes (East of Algiers). The identification of the plant was made by Professor Abdelkarim, head of the herbarium room at the level of the botanical department of the National Higher School of Agronomy (ENSA). The plant material is dried for a week in the open area and protected from light. The dried pods are then cut into small pieces and then reduced to a fine powder using an electric grinder. The powder obtained is stored away from air and humidity, in hermetically sealed glass jars.

2. Extraction of polyphenols

For this, 10 g of the powder of the pods of Sophora japonica are vigorously stirred in 100 ml of a methanolic solution (80 ml methanol/20 ml distilled water) using a vortex for 48 hours at room temperature. After filtration of the hydro-alcoholic mixture under a muslin cloth then on filter paper, the methanol is evaporated under reduced pressure (45°C) and the recovered extract is stored at 4°C until use. The yield of methanolic extracts is determined according to the following formula : $R\ \% = \frac{\mathrm{m}-m0}{\mathrm{mT}} \times 100$

m : the mass of the flask after evaporation ; m0: the mass of the empty balloon; mT: the total mass of the vegetable powder used in the extraction.

3. Quantitative analysis of phenolic compounds

*-Determination of polyphenols:*The polyphenol content is estimated by the folin-ciocalteu method [10]. 1ml of the methanolic extract of the plant is mixed with 1ml of Folin-Ciocalteu (2M) diluted 10 times and 1ml of sodium carbonate (Na_2CO_3) at a concentration of 75g/L. Absorbance is measured at 765nm, after incubation for 1 hour at room temperature in the dark. The calibration curve is carried out with gallic acid, following the same steps of the assay. The polyphenol content is expressed in milligrams of gallic acid equivalent per gram of dry weight of the plant (mg EAG/g Ps).

-Determination of flavonoids : Quantification of the flavonoid content in the polyphenolic extract of *Sophora japonica* is carried out by the $AlCl_3$ method [11]. 1ml of the plant extract is mixed with 1ml of aluminum trichloride solution ($AlCl_3$) (20 mg/ml) and a drop of acetic acid. The absorbance is measured at 415 nm after incubation at room temperature for 40 min. The flavonoids were quantified according to a calibration curve carried out by a standard flavonoid, quercetin (0.1 mg / ml). The flavonoid content is expressed in milligrams equivalent of quercetin per gram of dry weight of the plant (mg EQ/g Ps).

-Determination of condensed tannins : A dose of 50 µl of suitably diluted extract is added to 3 ml of 4% vanillin and 1.5 ml of concentrated sulfuric acid (H_2SO_4). After homogenization, the mixture is incubated at room temperature for 15 minutes. The absorbance of this preparation is measured at 500 nm. A calibration curve is carried out in parallel under the same operating conditions using catechin at concentrations of 0 to 400 µg/ml. The contents of condensed tannins are expressed in milligrams of catechin equivalent per gram of dry vegetable matter (mg EC.g-1 MS) [12].

-Determination of anthocyanins : The anthocyanin content estimation method uses two buffer systems: potassium chloride (KCl) at 0.025 M (pH=1.0) and sodium acetate ($C_2H_3NaO_2$) at 0.4 M (pH=4.5) [13]. 0.4 ml of extract is mixed with 3.6 ml of each buffer solution. The absorbances are measured at 510 and 700 nm. The absorbance which corresponds to anthocyanins is calculated as follows:

$$A = (A510 - A700)pH1 - (A510 - A700)pH2$$

The anthocyanin concentration, expressed in mg equivalent of cyanidine-3-glucoside per 100 g of the final weight, is calculated according to the following equation:

$$[Anthocyane] = \frac{A \times MM}{AM} \times 100$$

A: absorbance; MM: molecular mass of cyanidin-3-glucoside (449.2 g / mol); AM: coefficient of molar absorbance (26,900 mol.cm.L).

-Determination of Carotenoids : The estimation of the total carotenoid content contained in the extracts is carried out according to the following method [14]. 2 g of sample are homogenized with 20 ml of the following solvent mixture: (hexane/acetone/ethanol, 2: 1: 1). After stirring for 30 min, the upper phase is recovered and protected from light by aluminum foil. 10 ml of hexane are added and a second extraction is carried out. The mixture of the two extractions is centrifuged for 5 min at 6500 rpm. The carotenoid content is determined by measuring the absorbance of the hexane extract at 420 nm. Carotenoid concentrations are estimated by referring to the calibration curve obtained using β-carotene as the calibration standard. The concentrations are expressed in mg equivalent of β-carotene per 100g of the final weight (mg Eq β-carotene/100g of Fw).

4. Antioxidant activity evaluation

-Anti-radical power by the DPPH method : The anti-free radical activity of the different extracts is determined by the method using DPPH (2,2-Diphenyl-1-picrylhydrazyl) as a relatively stable free radical [15]. The DPPH solution is prepared by dissolving 2.4 mg of DPPH in 100 ml of methanol. Solutions of the phenolic extract of S. japonica at different concentrations are prepared in absolute methanol. 25 µl of the different solutions tested are added to 975µl of the DPPH solution. After shaking, the tubes are placed in the dark for 30 min. The reading is carried out by a spectrophotometer at 517 nm. The anti-radical activity or the antioxidant power is estimated according to the equation below:

$$A\% = \frac{A0 - AT}{A0} \times 100$$

A%: Percentage of anti-radical activity ; A0: Absorbance of the negative control (DPPH in methanol); AT: Absorbance of the sample.

-Calculation of IC50 : IC50 or 50% inhibitory concentration is the concentration of the tested sample necessary to reduce 50% of the DPPH radical. The IC50s are calculated graphically by the linear regressions of the plotted graphs, percentage of inhibition as a function of different concentrations of the fractions tested [16].

-Ferric reducing antioxidant power : 1 ml of the extract is mixed with 1 ml of a phosphate buffer solution (0.2 M, pH=6.6) and 1 ml of a potassium ferricyanide solution K3F (CN) 6 to 1. The whole is incubated in a water bath at 50°C for 20 min then 1 ml of trichloroacetic acid is added to stop the reaction.

The tubes are centrifuged at 3000 rpm for 10 min. 2 ml of the supernatant are mixed with 2 ml of distilled water and 0.4 ml of a 0.1 ferric chloride solution. The absorbance of the reaction medium is read at 700 nm [17]. The positive control is represented by a solution of standard antioxidants, quercetin and rutin whose the absorbance of which was measured under the same conditions as the sample. The sequestering effect of the samples against iron is expressed as a percentage of chelation according to the following equation:

$$\%\ chélation = \frac{Abs\ 700\ controle - Abs\ 700\ sample}{Abs\ 700\ controle} \times 100$$

-β- carotene bleaching test : The solution of β-carotene / linoleic acid is prepared by dissolving 0.5 mg of β-carotene in 1 ml of chloroform. 25 µl of linoleic acid and 200 mg of soluble Tween 40 are added. Once the chloroform has completely evaporated at 70°C, 100 ml of distilled water saturated with oxygen is added. To 2.5ml of this emulsion, 350µl of the methanolic extract or the reference antioxidants (quercetin, rutin and BHT) dissolved in methanol (2mg/ml) are added [18]. The discoloration kinetics of the solution in the presence and in the absence of antioxidant (negative control in which the sample is replaced by 350 µl of methanol) is monitored at 490 nm at regular time intervals for 48 hours.

-Evaluation of antioxidant activity by the ABTS method: Reduction of ABTS radicalation: The solution of the cationic radical ABTS•+ was prepared by mixing 2.45mM ABTS with 7mM potassium persulfate ($K_2S_2O_8$). After 16 hours of incubation, the ABTS+solution was diluted with methanol. A volume of 50µl of extract is added to 1 ml of the freshly prepared ABTS solution. The absorbance is read at 734nm [19]. ABTS scavenger activity is calculated by the following equation:

$$\textbf{ABTS radical scavenging activity}\ (\%) = \left(\mathbf{A0} - \frac{\mathbf{A}}{\mathbf{A0}}\right) \times \mathbf{100}$$

A0: absorbance of the negative control, A: absorbance of the sample

-Determination of total antioxidant capacity "phosphomolybdate test": The total antioxidant capacity of the extract is evaluated by the phosphomolybdenum method [20].

This technique is based on the reduction of molybdenum Mo (VI) present in the form of molybdate ions MoO_4^{2-} to molybdenum Mo (V) MoO^{2+} in the presence of the extract to form a green phosphate/Mo (V) complex at pH acid. The absorbance of the medium is determined at 695nm. 100µl of the sample is mixed with 1 ml of the reaction solution composed of H_2SO_4 (0.6M), phosphate buffer (28 mM) and $MoNH_4$ (4 mM). The tubes are then incubated for 90 min at 95°C. After incubation and cooling of the samples at room temperature, the absorbance is determined at 695nm against a blank. The total antioxidant capacity is expressed in milligram equivalent of ascorbic acid per gram of dry matter (mg EAA/g DM).

5. Statistical analysis

The results are expressed as mean±Standard Error at Mean (M±ESM) of 5 rats per group. Statistical analysis is performed using Statistica software (version 6, Genistat Conseils Inc., Montreal). After analysis of variance, the comparison of averages is performed by the student's t-test for paired samples. At a 95% confidence interval a $p \leq 0.05$ value is considered statistically significant.

RESULTS

1. Extraction and characterization of phenolic compounds

1.1. Yield of methanolic extract

The results of the extraction yield are reported in Table 1. The yield designates the mass of the extract determined after evaporation of the solvent, it is expressed as a percentage relative to the initial mass of the plant subjected to the extraction. The preparation of the extract from the powder of the pods of *Sophora japonica* L. was carried out with a polarity solvent, it is methanol. This extraction made it possible to obtain a methanolic extract with a yield calculated from the mass of the extract before and after evaporation relative to the vegetable powder.

Table 1**.** Yield of the extraction of the polyphenolic extract.

Extract	Extraction efficiency (%)
Methanolic extract	66,50%

1.2. Quantitative analysis of the methanolic extract

The objective of this analysis is to determine the content of different phenolic compounds. The main reason for choosing these substances is that most of the pharmacological effects of

plants are attributed to them. The results of the content of the extract in polyphenols, flavonoids, condensed tannins, anthocyanins and carotenoids are shown in Table 2.

Table 2. Determination of phenolic compounds.

Phenolic compounds	Concentrations
polyphenols	0.011 ± 0.02 mg EAG/g
flavonoids	15.65± 1.89 mg EQ/g
Condensed tannins	0.038 ± 0.11 mg ET/g
anthocyanins	0.085 ± 0.037 mg/ml
carotenoids	1.333±0.026 mg Eβ-carotène/g

The quantification of phenolic compounds was made according to a linear calibration curve (y = ax + b) at different concentrations and expressed in milligrams of gallic acid equivalent per gram of dry matter (mg EAG/g). It can be seen from the results in the table above that the amount of phenolic compounds is 0.011±0.02 mg EAG/g of extract, which shows that our extract is moderately rich in polyphenols. The flavonoid content of the extract was calculated from the standard curve expressed in milligrams equivalent to Quercetin per gram of dry matter (mg E /g). On remarque d'après les résultats que la quantité des flavonoïdes est de 15,65±1,89 mg EQ/g de la matière végétale. Ces résultats suggèrent que la plante *S. japonica* est extrêmement riche en flavonoïdes. The quantification of the tannins was carried out with reference to the calibration curve carried out using catechin as a positive control and expressed in equivalent milligrams of catechin per gram of dry vegetable matter (mg EC / g). The results obtained show that the level of tannins is 0.038±0.01 mg TE/g of plant material.

These results suggest that our extract is moderately rich in condensed tannins. The anthocyanin content of the methanolic extract of the plant studied is estimated using the differential pH method. The results of our study show that the anthocyanin content of the methanolic extract of *Sophora japonica* pods is 0.085 ± 0.037 mg / ml. Carotenoid determination requires the establishment of a calibration curve beforehand using β-carotene as a standard and expressed in equivalent milligrams of β-carotene per gram of extract (mg EQ of β-carotene/g of extract). The carotenoid content for the extract of S. japonica pods is 1.333±0.026 mg E β-carotene / g of extract, this value has a high carotenoid content in the extract.

2. Infrared characterization

The results of the analysis of the methanolic extract by infrared are illustrated in figure 1 and mentioned in Table 3. In the phenolic pod extract of *S. japonica*, the wide band around 3133.74 cm-1 is associated with the vibration of elongation of the O-H bond (of phenol function). The one having 3076.53 cm-1 corresponding to the vibration of elongation of the bond = CH (anhydride function) the average band located at 1804.48 cm-1 corresponds to the vibration of elongation of bond CO (of anhydride function) and those who have vibrations of elongations of the order of 1632.86 cm^{-1} and 1480.43 cm^{-1} 1178.58 cm^{-1} with CC CC, C-OH bonds of the phenol, aromatic and alcohol functions respectively. The weak band 959.822 cm-1 corresponds to the vibration of elongation of the link R-CH = CH_2 (of Alcene function).

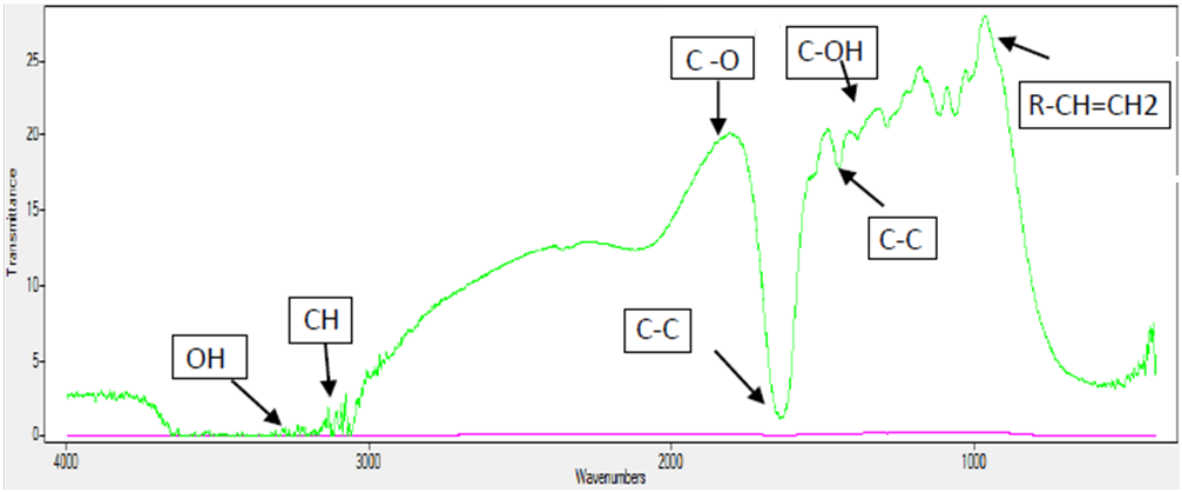

Figure 1. Infrared chromatogram of methanolic extract in *Sophora japonica* pods.

Table 3. Result of the infrared analysis.

Methanolic extract of *S. japonica*	Wavelength cm^{-1}	Connection	Function	Nature of the connection
Phenolic compounds	3133.74	O-H	Phenol	hydrogen bonds
	3076.53	=C-H	Alkene	
	1804.48	C-O	Anhydrides	
	1632.86	C-C	Phenol	
	1480.43	C-C	Aromatic	
	1178.58	C-OH	Alcohol	
	959.822	R-CH=CH_2	Alkene	

3. Evaluation of antioxidant capacity

3.1. DPPH free radical scavenging method

Anti-free radical activity was estimated by following the reduction in DPPH to 517 nm. On the basis of the data which represent the anti-radical power as a function of the different concentrations of the standards, the results are expressed in figure 2. The figure illustrates the effectiveness of methanolic extract, BHT and ascorbic acid in trapping the DPPH radical, expressed by the inhibition rate (I%) or the percentage of anti-radical activity depending on the different concentrations in the reaction medium. The percentage of antioxidant activity gradually increases. It is a proportional relationship. At a concentration of 1 mg / ml, the percentages of the anti-free radical activity of the standards and of the extract of the plant tested sophora japonica are respectively of the order of 82.74%±0.84 (BHT) and 73.60%±4.26 (Ascorbic acid), 96.47%±0.26 (methanolic extract). Thus, it is found that the methanolic extract has an anti-radical activity greater than that of the standards (BHT and ascorbic acid).

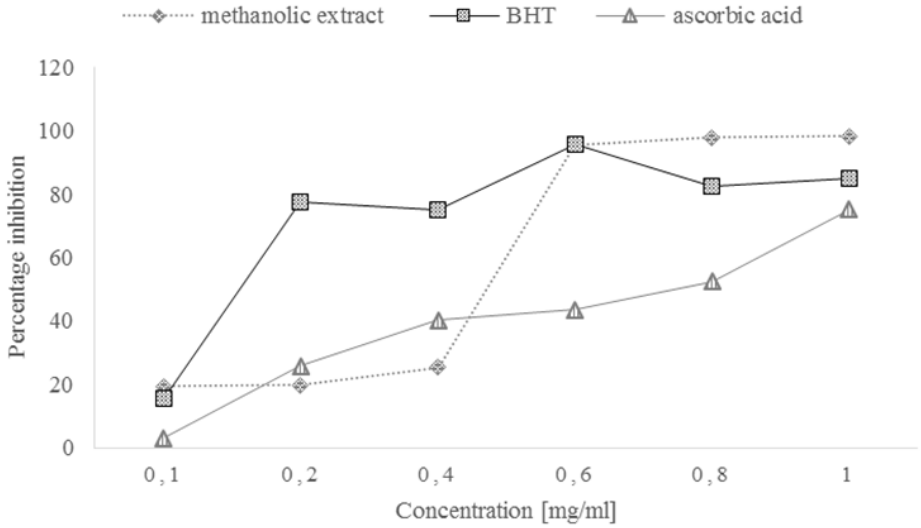

Figure 2. Anti-free radical activity of the methanolic extract of the plant *Sophora japonica* L. BHT and ascorbic acid are used as positive controls. Each point represents the mean±E.S.M (mean of 3 tests).

The results obtained also made it possible to determine the values of IC50 (the value which corresponds to 50% inhibition) in order to be able to evaluate the activity of the extract.

The IC50 of the extract and of the standards were determined graphically from the trend curves representing the inhibition rates. The different IC50s are shown in table 4.

Table 4. The IC50% values for the methanolic extract of the plant, ascorbic acid and BHT

Sample	IC50 mg/ml
Methanolic extract	0,76 ±0,12
Ascorbic acid	0,54±0,11
BHT	0,43±0,005

The value of IC50 is inversely proportional to the antioxidant capacity of a compound, because it reflects the amount of antioxidant required to neutralize 50% of the initial concentration of the free radical in the medium. The table shows that the overall anti-radical potential of the tested extract is greater than that of ascorbic acid and BHT whose IC50 is 0.76±0.12 mg/ml, followed by ascorbic acid 0.54±0.11 mg / ml then BHT 0.43±0.005 mg/ml. The lower the IC50 value, the more appreciable the anti-radical activity of a compound.

3.2. Ferrous iron chelation

The results of the antioxidant activity of the methanolic extract by the ferrous ion chelation method are shown in Table 5. The results obtained show that the capacity of the extract which has the percentage of reducing Iron is of the order of 78.96%±0.01. These results are much higher than those of quercetin and rutin which have a chelation percentage of around 17.55%±0.02 and 13.87%±0.015 respectively.

Table 5. Chelating activity of ferrous ions by the methanolic extract, quercetin and rutin.

Extrait	Pourcentage de chélation (%)
Methanolic extract	78.96±0.01
Quercetin	17.55±0.02
Rutin	13.87±0.015

3.3. β-carotene bleaching test

The presence of antioxidants could neutralize free radicals derived from linoleic acid and therefore prevent the oxidation and bleaching of β-carotene.

The whitening kinetics of β-carotene in the presence and absence of the extract of *Sophora japonica* L., and of the standards (quercetin, rutin and BHT) are shown in figure 3.

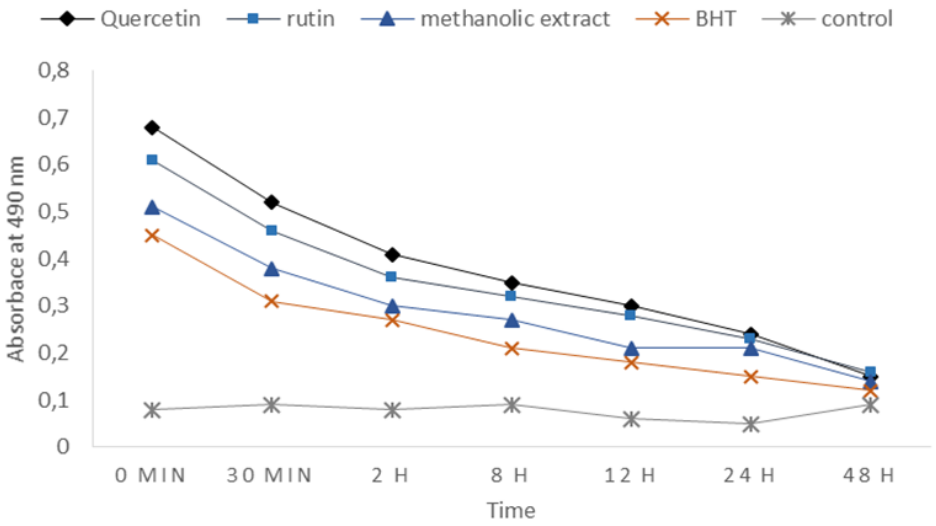

Figure 3. Whitening kinetics of β-carotene at 490 nm in the presence and absence of the methanolic extract of *Sophora japonica* L., quercetin, rutin, BHT and the control. Quercetin, rutin and BHT are used as positive controls. Each point represents the mean ± E.S.M (mean of 3 tests).

The antioxidant potential of the extract and standards is evaluated by determining the inhibition capacity of the oxidation of β-carotene. The figure above shows oxidation by bleaching β-carotene over 48 hours. The decrease in absorbance is due to the oxidation of β-carotene and linoleic acid. The addition of antioxidant substance plays a protective role and prevents or slows down this oxidation. This protective effect is measured by the rate of inhibition of β-carotene bleaching at the end of the incubation period. From these results, it is obvious that the extract of *S. japonica* and the standards tested are capable of inhibiting the coupled oxidation of linoleic acid and of β-carotene compared to the negative control which represents a weak inhibition of β-carotene bleaching.

3.4. Reduced Radical cation ABTS

The results of the extract and of the standard analyzed are expressed as a percentage of inhibition and are represented in figure 4. It follows that the activity of anti-free radical ABTS is all the more higher as the concentration is high. On the other hand, these results indicate that for all concentrations, the extract has the greatest inhibitory effect than the BHT standard. At the minimum concentration of 0.1 mg / ml, the percentage inhibition of the extract and of BHT is 92.12%±3.829 and 91.14%±0.3 respectively. At a concentration of 0.4mg/ml, the percentage inhibition is 98.23%±1.202 and 94.47%±0.266 respectively. These results clearly show that the methanolic extract of S. japonica has an interesting antioxidant property.

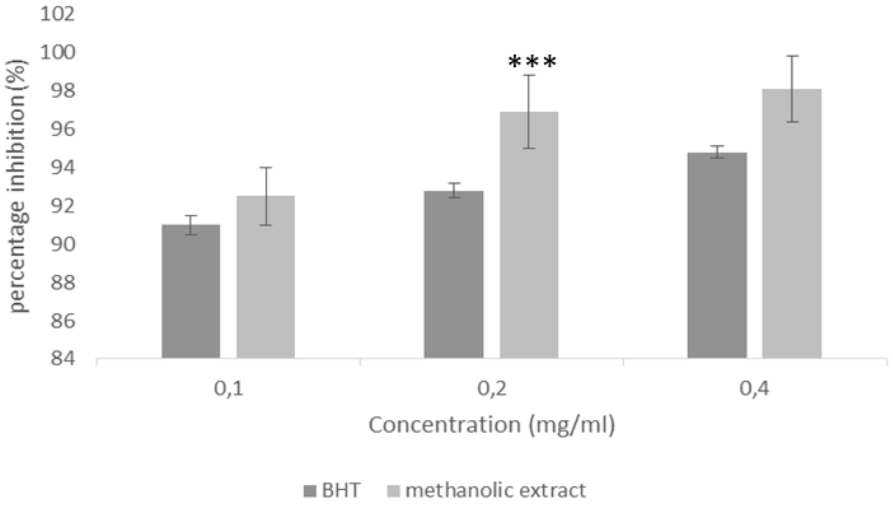

Figure 4. Histogram representing the anti-free radical activity of the methanolic extract and of BHT. Each point represents the Average ± E.S.M (average of 3 tests). *** p <0.001 the difference is very highly significan.

3.5. Total antioxidant activity "phosphomolybdate test"

The total antioxidant capacity of the methanolic extract is expressed in mg equivalent of ascorbic acid per g of the dry extract (mg EAA/g of extract). The results are illustrated in the figure and shown in table 6. The total antioxidant capacity of the methanolic extract is expressed in number of equivalent of ascorbic acid from the standard curve (y=-37.03x+84.10, R^2=0.989). The results of the total antioxidant capacity of the methanolic extract is 0.113 mg EAA/g of the dry extract. This concentration shows that our extract expresses an antioxidant power which can be mainly due to the richness of the extract in polyphenols, particularly the flavonoids.

Table 6. Rate of the antioxidant capacity of the methanolic extract.

Extract	Concentration in mg equivalent of ascorbic acid per g of the dry extract
Methanolic extract	0.113±0.0002

The values are represented as Average ± ESM (values of 3 tests).

DISCUSSION

The method of extraction adopted, based on the differential solubility of polyphenolic compounds in organic solvents, made it possible to extract the maximum of compounds and to prevent their denaturation or modification likely due to the high temperatures used in other extraction methods. In our work, the yield obtained is 66.50%. Our results are far from a study where the yield of the methanolic extract of the plant *Rhamnus alaternus* L. (Fabacea) is 11% [21]. The difference in yield between the extracts is due to the extraction techniques used, which are completely different and to the chemical composition which differs from one extract to another [21]. In a study carried out on Rhamnus alaternus L. from Tunisia, similar results were found. Maceration of the leaves in methanol gave a yield of 9% [22].

It is interesting to determine the concentration of the various polyphenols contained in the extract of S. japonica and which are likely to act on the pharmacological effects observed. According to the results obtained, the quantity of phenolic compounds obtained is 0.011±0.02 mg EAG / g of extract, that of flavonoids is 15.65±1.89 mg EQ/g of vegetable matter, tannins total is 0.038±0.01 mg TE/g of plant material, anthocyanins of 0.085±0.037 mg / ml and finally carotenoids is 1.333±0.026 mg E β-carotene / g of extract. The polyphenol contents determined are not absolute measures of the quantities of phenols in the starting material. They are, in fact, based on the relative reducing capacity equivalent to each standard used. The values obtained by the colorimetric method provide direct information on the quantity of antioxidant phenolic groups in the extract which depends essentially on the number of hydroxyl groups in these [23]. The polyphenolic profile of plant extracts can vary under the influence of various factors including variety, climate, geographic location, different diseases that can affect the plant, plant maturity, temperature and solvent extraction [24]. The antioxidant activity of the extract was assessed in vitro by five different methods. These are the trapping of the free radical DPPH, the whitening system of β-carotene, the reducing power of ferrous iron (FRAP), the trapping of the cationic radical $ABTS^+$ and the total antioxidant capacity. The results obtained show that the overall anti-free radical potential of the tested extract is greater than that of ascorbic acid and BHT whose IC50 is 0.76±0.12 mg/ml, followed by acid ascorbic 0.54±0.11 mg/ml then BHT 0.43±0.005 mg/ml. These results are not in agreement with the results of several publications, which have reported a positive correlation between all phenolic content and antioxidant activity [25 ; 26]. Indeed, it has been shown that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins reduce and discolor DPPH due to their ability to yield hydrogen [27]. The polyphenols in the methanolic extract of S. japonica are probably responsible for the antioxidant activity. Some studies have shown a good correlation between IC50 and the content of polyphenols and flavonoids [28, 29]. Furthermore, it is well established that antioxidant activity is positively correlated with the structure of polyphenols. Generally, polyphenols with a high number of hydroxyl groups have the highest antioxidant activity [30]. Due to their power to give more atoms to stabilize free radicals [31]. This may explain in part that the anti-radical activity is dependent on the number, the position and the nature of the substituents on the B and C rings (hydroxyl, metaxylated, glycosylated groups) and the degree of polymerization [32]. Thus, the antioxidant effect is not only dose-dependent but also structure-dependent [33]. The results obtained in the ferrous iron chelation method show that the capacity of the extract which has the percentage of reducing Iron is of the order of 78.96%±0.01. The presence of reducing agents in plant extracts causes the reduction of Fe^{3+} / Fe^{2+} Ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be assessed by measuring and monitoring the increase in density of the blue color in the reaction medium at 700nm [27]. The reducing power of plant extracts is probably due to the presence of hydroxyl groups in phenolic compounds which can serve as an electron donor. Antioxidants are therefore considered to be reducers and inactivators of oxidants [27]. Thus, quercetin which combines all these substituents is a particularly effective metallic complexing agent [26]. This autoxidation process depends on multiple parameters such as the concentration of metal ion and polyphenols, temperature, pH, the presence of complexing agents [26]. Peroxide radicals originating from the trapping of hydrogen atoms from the diallyl methylene groups of linoleic acid attack the highly unsaturated β-carotene thus causing its oxidation and therefore the disappearance of the orange color [34]. However, the presence of antioxidants in the reaction mixture could relatively inhibit the oxidation of β-carotene. This inhibition is probably due either to the inhibition of the auto-oxidation of linoleic acid or to the trapping of peroxide radicals formed during their oxidation [35]. Quercetin and rutin exhibited the greatest inhibition, followed by methanolic extract and BHT. Indeed, a remarkable and significant linear correlation is found between the AAR of the extracts and the flavonoid content (p<0.05). These results are probably due to the high specificity of the β-carotene bleaching test for lipophilic compound [36]. Indeed, an extract that inhibits or delays β-carotene bleaching can also be described as a free radical scavenger and as a primary antioxidant [37]. The inhibition of oxidation of linoleic acid coupled with that of β-carotene seems very useful as a mimetic model of lipid peroxidation in biological membranes [38].

The antioxidant activity determined by the method using the ABTS radical in the extract and BHT varies significantly. In fact, at a concentration of 0.4 mg / ml, the percentage inhibition of the extract of *S. japonica* is 98.23%±1.202. These results are superior to those of Godevac et *al.* [39] who found an anti-free radical potential ($ABTS^{+\bullet}$) around 14.06%±23.08 for the methanolic extract of the plant *Anthyllis aura* (Fabaceae). In addition, the antioxidant activity of our species is higher than that of *Anthyllis vulneraria* 29.52%±0.81 and lower than that of *Coronilla emerus* 107.17%±2.43 (Fabaceae) [39]. The phosphomolybdate test is widely used and adequate to determine the total antioxidant activity (AAT) of plant extracts. Therefore, we applied it in this study to determine the AAT of the methanolic extract of S. japonica. According to the results obtained, the effectiveness of the samples in reducing Mo^{+6} to Mo^{+5} is variable. The reducing power of ammonium phosphomolybdate depends on the content of phenolic compounds in the samples and the position as well as the number of hydroxyl groups. Scalbert [40] has shown that this difference may be due to the existence of polyphenols which could be linked to the degree of oxidation.

Indeed, the latter are components very susceptible to auto-oxidation in the presence of oxygen in the air [41; 42] report that most Fabaceae have total antioxidant activity due to the presence of polyphenols. However, the phenolic fraction does not incorporate all of the synergistic antioxidant activities between the antioxidants in a mixture. This is due to the fact that antioxidant activity depends not only on the concentration but also on the structure and nature of these antioxidants.

CONCLUSION

All of these results obtained open up prospects for the use of Sophora japonica for various uses and constitute only a start in the field of research for biologically active natural substances. Additional tests will be necessary in order to confirm the activities highlighted.

REFERENCES

[1]. Marouf, A. & Joël, R. (2007). La botanique de A à Z. Edition Dunod Paris: p. 66-82.

[2]. Menichini F., Loizzo MR., Bonesi M., Conforti F., De Luca D., Statti GA., de Cindio B., Menichini F., Tundis R. (2011). Phytochemical profile, antioxidant, anti-inflammatory and hypoglycemic potential of hydroalcoholic extracts from *Citrus medica* L. cv Diamante flowers, leaves and fruits at two maturity stages. Food and Chemical Toxicology **49**, p. 1549-1555.

[3]. Djeddi S., Yannakopoulou E., Papadopoulos K., et Skaltsa, H. (2015). Activités anti-radicalaires de l'huile essentielle et des extraits bruts de *Thymus numidicus* Poiret en Algérie. Afrique Science **11**(2), p. 58 – 65.

[4]. Galeotti F., Barile E., Curir P., Dolci M., Lanzotti V. (2008). Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. Phytochemistry Letters **1**(1), p. 44-48.

[5]. Bruneton, J. (1999). Pharmacognosie, phytochimie et plantes médicinales. La Voisier Tec et Doc Paris**,** 5ème édition, p. 250-270.

[6]. Rolland MF. (2004). Morphologie et alimentation de l'enfant : Évolution au cours des dernières décennies. Cahiers de Nutrition et de Diététique **39**(3), p. 178-184.

[7]. Moon JK. & Shibamoto T. (2009). Antioxidant Assays for Plant and Food Components. *Journal of Agriculture and Food Chemistry* **57**(5), p. 1655-1666.

[8]. Zellagui A., Gherraf N., Ladjel S., Hameurlaine S. (2012). Chemical composition and antibacterial activity of the essential oils from *Launaea resedifolia* L. Organic and Medicinal Chemistry Letters **2**(1), p.31.

[9]. Tadeg H., Mohammed A., Asres K., and Gebre-Mariam T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology **100**(1-2), p. 168–75.

[10]. Li J., Jiang Y. (2007). Litchi Flavonoids: Isolation, Identification and Biological Activity. Molecules **12**(4), p.745-758.

[11]. Kumaran R., Karunakaran J. (2006). Antioxidant Activities of the Methanol Extract of *Cardiospermum halicacabum*. Pharmaceutical Biology **44**(2), p.146-151.

[12]. Sun B., Ricardo-da-Silva, M., Spranger I. (1998). Critical Factors of Vanillin Assay for Catechins and Proanthocyanidins. Journal of Agriculture and Food Chemistry **46**(10), p. 4267-4274.

[13]. Çam M., Hisil Y. et Durmaz G. (2009) Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. Food Chemistry **112**, p. 721-726.

[14]. Yolanda Salinas M., Salvador Saavedra A., Jesús Soria R., Edgar Espinosa T. (2008). Physicochemical characteristics and carotenoid content in yellow corn (*Zea mays* L.) grown in the State of Mexico. Agricultura técnica en México **34**(3), p. 357-364.

[15]. Mansouri A., Embarek G., Kokkalou E., Kefalas P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chemistry **89**(3): p. 411-420.

[16]. Marxen K., Klaus HV., Sebastian L., Ralf H., Andreas R., and Ulf-Peter H. (2007). Determination of DPPH Radical Oxidation Caused by Methanolic Extracts of Some Microalgal Species by Linear Regression Analysis of Spectrophotometric Measurements. Sensors (Basel). **7**(10), p. 2080–2095.

[17]. Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 44, p. 307-315.

[18]. Kartal N., Sokmen M., Tepe B., Daferera D., Polissiou M., Sokmen A. (2007). Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. Food Chemistry, 100, p. 584-589.

[19]. Djeridane A., Yous M., Nadjemi B., Boutassouna D., Stocker P., Vidal N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, **97**(4), p. 654-660.

[20]. Prieto P., Pineda M., Anguilar M., (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anale of Biochemistry **269**(2), p. 337-341.

[21]. Harrar, AN. (2012). Activités antioxydante et antimicrobienne d'extraits de *Rhamnus alaternus L*. Thèse de doctorat, Université Ferhat Abbas de Sétif, Algérie, p.73.

[22]. Ben Ammar R., Kilani S., Bouhlel I., Ezzi L., Skandrani I., Boubaker J., Ben Sghaier M., Naffeti A., Mahmoud A., Chekir-Ghedira L. and Ghedira K. (2008). Antiproliferative, Antioxidant, and Antimutagenic Activities of Flavonoid-Enriched Extracts from (Tunisian) *Rhamnus alaternus* L.: Combination with the Phytochemical Composition. Drug and Chemical Toxicology **31**(1), p. 61-80.

[23]. Balasundaram C., & Bhuvaneswari R. (2009). Anti-bacterial activity of *Acorus calamus* and some of its derivates against fish pathogen *Aeromonas hydrophila*. Journal of Medicinal Plants Research **3**(7), p. 538-547.

[24]. Sousa C., Taveira M., Valentão P., Fernandes F., Pereira J.A., Estevinho L., Bento A., Ferreres F., Seabra R.M., Andrade P.B. (2008). Inflorescences of Brassicacea species as source of bioactive compounds: A comparative study. Food Chemistry **110**(4), p. 953-61.

[25]. Kadri H., Djilani SE., Djilani A. (2013). Phytochemical constituents, antioxidant activity, total phenolic and flavonoid contents of *Arisarum Vulgare* seeds.. *Acta Scientiarum Polonorum Technologia Alimentaria* **12**(2), p. 169-173.

[26]. Ghedadba N., Hambaba L., Ayachi A., Aberkane MC., Bousselsela H., Oueld-Mokhtar SM. (2015). Polyphénols totaux, activités antioxydante et antimicrobienne des extraits des feuilles de *Marrubium deserti* de Noé. Phytotherapie **13**(2):118-129.

[27]. Bougandoura N., Bendimerad N. (2013). Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de *Satureja calamintha* ssp.Nepeta (L.) Briq. Nature & Technologie 09, p. 14-19

[28]. Athamena S., Chalghem I., Kassah-Laouar A., Laroui S., et Khebri S. (2010). Activite anti-oxydante et antimicrobienne d'extraits de *Cuminum cyminum* L. Lebanese Science Journal 11 (1), P. 69-81.

[29]. Mariod, AA., Siti FF., Maznah, I. (2010). Preparation and characterisation of protein concentrates from defatted kenaf seed. Food Chemistry **123**(3), p.747-752.

[30]. Heim K., Tagliaferro AR., Bobilya DJ. (2002). Flavonoids antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry 13, p. 572-584.

[31]. Torres A., de Pinedo P., Peñalver Morales JC. (2007). Synthesis and evaluation of new phenolic-based antioxidants: Structure–activity relationship. Food Chemistry **103**(1), p. 55-61.

[32]. Popovici C., Saykova I., Tylkowski B. (2009). Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. Revue de génie industriel **4**, p. 25-39.

[33]. Rodríguez-Bernaldo A. de Quirós MA., Lage-Yusty J., López-Hernández. (2010). Determination of phenolic compounds in macroalgae for human consumption. Food Chemistry **121**(2), p. 634-638.

[34]. Amarowicz R., Pegg RB., Rahimi-Moghaddamc P., Barl B. et Weil JA. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry **84**, p. 551–562.

[35]. Tepe B., Daferera D., Sokmen A., Sokmen M., Polissou M. (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chemistry ***90***, p. 333-340.

[36]. Gachkar L., Yadegari D., Rezaei MB., Taghizadeh M., Astaneh SA., Rasooli I. (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chemistry **102**, p. 898-904.

[37]. Liyana-Pathirana CM, Shahidi F. (2006). Antioxydant propreties of commercial soft and hard winter wheats (*Triticum aestivium* L.) and their milling fractions. Journal of the Science and Food Agriculture **86**, p. 477-485.

[38]. Ferreira I., Barros L., Soares ME., Bastos ML., Pereira JA. (2007). Antioxidant activity and phenolic contents of *Olea europaea* L. leaves sprayed with different copper formulations.Food Chemistry **103**, p.188-195.

[39]. Godevac D., Zdunić G., Šavikin K., Vajs V., & Menković N. (2008). Antioxidant activity of nine Fabaceae species growing in Serbia and Montenegro. Fitoterapia **79**, 185-187.

[40]. Scalbert A. (1991). Antimicrobial properties of tannins. Phytochemistry **30**(12), 3875-3883.

[41]. Wojdyło A., Jan O., Renata C. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry **105**(3), 940-949.

[42]. Falah S., Suzuki T., Katayama T. (2008). Chemical constituents from Swietenia macrophylla bark and their antioxidant activity. Pakistan Journal of Biological Science **11**, p. 2007-2012.