Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(22):1-6
(http://www.derpharmachemica.com/archive.html)

Determination of Primary and Functional Metabolites of *Salvia argentea* and Evaluation of its Leaves and Roots Antioxidant Activity

Wafaa Tehami[1], Asmaa Kherraf[1], Wahiba Boufeldja[1], Mustapha Mahmoud DIF[3], Amira Ghislaine DRA[1], Bouziane Abbouni[2], Mohammed Benali[1]

[1]Biotoxicology Laboratory Department of Biology, University of Djilali Liabes, Sidi Bel Abbès 22000, Algeria

[2]Molecular Microbiology and Proteomic Laboratory, Department of Biology, University of Djilali Liabes, Sidi Bel Abbès 22000, Algeria.

[3]Sciences Institute, Department of Biology, University Center, El Bayadh, 32000, Algeria

---

ABSTRACT

The objective of this work is to determine the primary metabolites and functional principles and to evaluate the antioxidant activity of silver sage Salvia argentea, an herbaceous plant growing in Algeria. Physicochemical analyzes performed for the first time revealed appreciable levels of primary metabolites. It contains for the leaves and roots, respectively, a moisture content of 9.50 ± 0.01% and 8.63 ± 0.16%, ash content of 14.68 ± 0.10% and 9.03 ± 0.04% and for the fat content a rate of 2.92 ± 0.11% and 3.94 ± 0.05%. The aerial and subterranean parts of S. argentea, have respectively contents of 10.73 ± 0.18% and 5.23 ± 0.32% of proteins. On the other hand, poverty in sugars in the roots was observed (0.15 ± 0.12%) compared to leaves (4.13 ± 0.04%). The mean polyphenol contents of leaves and roots in methanolic and aqueous extracts were 87.13 ± 0.32-55.64 ± 0.35 mg GAE/g and 71.80 0.79-48.20 0.79 mg GAE/g. The flavonoids were 59.97 ± 0.21-31.22 ± 0.49 mg CE/g and 44.16 ± 0.60-18.12 ± 0.54 mg CE/g and tannins respectively of 3.70 ± 0.09-5.58 ± 0.13 mg CE/g and 4.01 ± 0.16-5.83 ± 0.09 mg CE/g. In addition, the extracts tested showed appreciable antioxidant powers using the FRAP iron reduction method and the free radical scavenging method DPPH. The IC50s range from 45.42 ± 0.46 to 416.12 ± 0.26 µg/ml. This study indicates that S. argentea may be a potential source of antioxidants used for therapeutic purposes.

Keywords: *Salvia argentea*, Primary metabolites, Functional principles, Antioxidant activity

---

INTRODUCTION

The discovery of the natural resources of the plant world remains crucial for the development of new therapeutic remedies. Secondary plant metabolites have been used for centuries in traditional medicine because of their large biological activities including antioxidant properties [1,2]. The essential oil of *Salvia argentea*, collected in the south-east of Serbia, was analyzed by GC-MS. Forty-seven constituents have been identified. The oil was characterized by a high content of sesquiterpenes. The main constituents were viridiflorol (32.4%), manol (14.6%) and α-humulene (10.7%) [3]. In recent years, plant extracts have appeared on the market as antioxidants used in the food and therapeutic industries [4]. The Lamiaceae family encompasses a large number of plants known for their antioxidant properties and the Salvia kind has about 900 species distributed widely throughout the world [5], of which 23 species grow in Algeria [6]. Apart from the studies carried out on its terpenoids by Couladis et al., Lakhel et al., and Riccobono et al., [3,7,8], *S. argentea* seems to have never been studied chemically unlike other species of the same genus as *S. officinalis*, *S. triloba*, *S. cavaleriei* [9,10]. For this purpose, we have chosen to determine the primary metabolites allowing biochemical characterization of the plant and a quantification of the functional principles of leaves and roots, namely polyphenols, flavonoids and tannins, and to evaluate the Antioxidant activity of some extracts.

MATERIALS AND METHODS

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), bovine serum albumin, sodium carbonate the Folin–Ciocalteu reagent, gallic acid, catechin, ascorbic acid, methano and iron chloride were purchased from Sigma–Aldrich (Germany).

Plant material

The leaves and roots of *Salvia argentea* were harvested in the region of Tenira (Sidi Bel-Abbès, Algeria) in April 2015. This plant was identified by the Department of Botany of the University of Sidi Bel-Abbès and was kept under the number SA/04.2015 in the laboratory of biotoxicology. The harvested plant material was dried away from moisture and light and then powdered with a crusher and stored in a brown bottle until use.

Preparation of the crude extracts by maceration and under reflux

The leaves and roots of *S. argentea* (10 g) powder were extracted for 24 h with 100 ml of 80% methanol at room temperature. The powdered plant material was contacted with water and the mixture was boiled under reflux for three hours [11]. The extracts obtained were filtered under vacuum and the various filtrates were concentrated to dryness by evaporation in a rotary evaporator (Heidolph instruments). The residues obtained were stored at 4°C.

Physicochemical analyzes

Moisture and ash levels were estimated using the methods described by Audigie et al., 1984 and AOAC, 1990 [12,13]. For fat content, samples (leaves and roots) were continuously extracted with boiling petroleum ether which gradually dissolved the fat using the Soxhlet extractor [14]. The total sugars were determined by dispersing 1 g of powdered sample in 10 ml of 25% (v/v) DMSO in water. The mixture was incubated in a boiling water bath for 15 min. 0.1 ml of this mixture was diluted in 9.9 ml of water. To 0.5 ml of the latter was added 0.5 ml of phenol (5%). After homogenization, 2 ml of H_2SO_4 (75%) were added. This mixture was incubated for 15 min in the water bath, then boiling for 15 min in the dark before reading on the spectrophotometer (Shimadzu Scientific Instruments) at 492 nm. Glucose was used as a standard in the calibration range [15]. For protein determination, the protein extract was obtained by adding 0.226 g of ammonium sulfate to 1 ml of the aqueous extract of the sample. After a 12 h cold incubation, the solution was centrifuged at 13400 g at 4°C for 20 min. The supernatant obtained was removed and the pellet taken up in phosphate buffer saline pH 7.4 and then stored at -20°C until assayed [16]. Proteins were assayed by the method of Lowry et al., [17]. Calibration was carried out with Bovine Serum Albumin (BSA) with a concentration range of 0 to 0.25 mg/ml.

Determination of phenolic compounds

Determination of total phenol content

The total phenol content of leaves and roots was determined spectrophotometrically using the Folin-Ciocalteu reagent [18]. 0.2 ml of each diluted extract or standard gallic acid was mixed with 1 ml of Folin-Ciocalteu reagent (diluted to 10^{th} with distilled water) and 0.8 ml of 7.5% Na_2CO_3. After 30 min of incubation, the absorbance was measured at 765 nm. The standard curve was carried out using a range of gallic acid concentrations ranging from 0 to 100 mg/L. The results were expressed in milligrams equivalent gallic acid per gram of dry matter (mg GAE/g MS).

Determination of total flavonoids

The aluminum chloride colorimetric method was used for the determination of flavonoids [19]. 0.5 ml of each extract diluted with 1.5 ml of distilled water was mixed with 0.3 ml of 5% $NaNO_2$. 3 ml of 10% $AlCl_3$ was added 5 min later. After 6 min, 1 ml of 4% NaOH was added. The solution was well mixed and the absorbance was measured at 510 nm. Catechin was used as the standard for the calibration curve. The total flavonoid contents were expressed in mg catechin equivalent/g dry matter (mg EC/g MS).

Determination of condensed tannins

The extracts of the various parts of the plant (0.1-0.5 ml) were placed in tubes to which 3 ml of a 4% (w/v) vanillin methanol solution were added. After vigorous stirring, 1.5 ml of concentrated HCl was immediately added and the mixture was stirred again. Absorbance was measured at 500 nm after 20 min incubation [20]. The calibration curve was prepared under the same conditions using catechin as standard and the results were expressed in mg catechin equivalent/g dry matter (mg EC/g MS).

DPPH radical scavenging activity

Evaluation of antioxidant activity of the leaves and roots of *S. argentea* was performed by the test 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the protocol recommended by Benhammou et al., 2009 [21] with a slight modification. A methanolic solution (50 µl) of each extract at different concentrations was added to 1.95 ml of the 25 mg/l of DPPH methanol solution. The absorbance was measured at 515 nm after 30 min of incubation in the absence of light and at 37°C. The antioxidant capacity (AC) of the sample in percent (%) was calculated according to the following equation:

$$AC\% = [Abs\ control - Abs\ extract)/Abs\ control] \times 100.$$

Ferric reducing antioxidant power (FRAP)

The iron reducing activity of the extracts was estimated according to the method of Oyaizu et al., [22]. 0.5 ml of each extract at

different concentrations was mixed with 1.25 ml of a 0.2 M phosphate buffer solution (pH=6.6) and 1.25 ml of a solution of 1% $K_3Fe(CN)_6$ potassium ferricyanide. The whole was incubated at 50°C for 20 min, and then cooled to room temperature. 2.5 ml of 10% trichloroacetic acid were added to stop the reaction and then the tubes were centrifuged at 3000 rpm for 10 minutes. 1.25 ml of the supernatant was added to 1.25 ml of distilled water and 250 µl of a 0.1% iron chloride ($FeCl_3$) solution. The absorbance was read by spectrophotometer (Shimadzu Scientific Instruments) at a wavelength of 700 nm. The positive control was represented by a solution of a standard antioxidant, ascorbic acid, the absorbance of which was measured under the same conditions as the samples.

Statistical analysis

The results are given as mean ± SD. The statistical evaluation was carried out using the StatView software with an ANOVA analysis followed by the t-test. The value of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Determination of primary metabolites

The results of the primary metabolites are shown in Figure 1. The water contents expressed as a percentage in the two dried parts (leaves and roots) of *S. argentea* were 8.63 ± 0.16% for the roots and 9.50 ± 0.01% for the leaves. According to Paris et al. [23], to ensure good preservation, the water content must be less than or equal to 10%. The studied plant contains a higher level of leaf ash as compared to the roots, with values of 14.68 ± 0.10% and 9.03 ± 0.04%, respectively. For the fat, the values were relatively low and, contrary to the ash content, the roots have a higher rate (3.94 ± 0.05%) than the leaves (2.92 ± 0.11%). The sugar content observed was 4.13 ± 0.04% in the leaves and only 0.15 ± 0.12% at the roots. The protein level determined at the leaf level was approximately twice that of the roots with values of 10.73 ± 0.18% and 5.23 ± 0.32%, respectively.

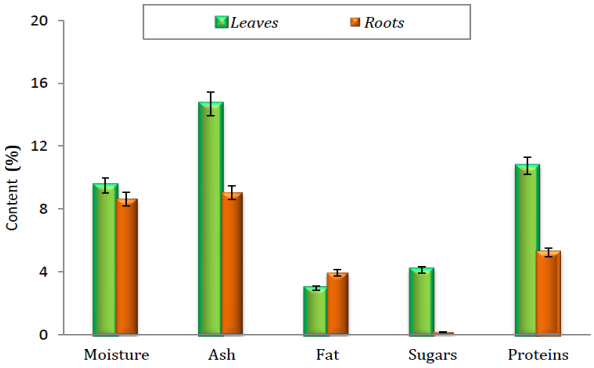

Figure 1: Levels of primary metabolites in leaves and roots of *Salvia argentea* (Values are expressed as mean ± standard deviations (n=3) with P<0.05 considered to be significant)

Determination of secondary metabolites

Total polyphenols

The total polyphenols, expressed as the gallic acid equivalent of the leaves and roots of *S. argentea*, were assayed using the Folin-Ciocalteu method [18]. The amounts of total polyphenols in the leaves and roots of *S. argentea* were shown in Table 1. The amount of total phenols was higher in the aerial part (MEL) 87.13 ± 0.32 mg GAE/g compared to that of the underground part (MER) 55.64 ± 0.35 mg GAE/g ($p<0.01$). These levels were also higher than those obtained in aqueous medium with contents of 71.80 ± 0.79 mg GAE/g and 48.20 ± 0.79 mg GAE/g for leaves and roots, respectively. The work of Ben Farhat et al., and Ben Farhat et al., [24,25] reported that the methanolic and aqueous extracts (distillation) of the aerial part of the same species growing in Tunisia had phenolic compound contents of 67.67 – 72.02 mg GAE/g and 41.47-48.90 mg GAE/g obtained by the distillation process. The work of Cuceu et al., [26] on methanol extracts of four species of Salvia, shows that the polyphenols determined by the Folin Ciocalteus method vary at most 27-37 mg GAE/g dry matter. According to Lamien-Meda et al., [27] the quantitative variations of polyphenols can be due to various factors, geographical, climatic, vegetative phase, genetic equipment and others. These factors may influence the accumulation of phenolic compounds by synthesizing different amounts and/or types of phenolic compounds [28]. According to Lee et al., [29], the extraction and quantification methods can also influence the estimation of the total phenol content.

Table 1: Secondary metabolites contents of leaves and roots of *Salvia argentea*

Extracts	Polyphenols[a]	Flavonoids[b]	Tannins[b]
MEL	87.13 ± 0.32	59.97 ± 0.21	3.70 ± 0.09
MER	55.64 ± 0.35	31.22 ± 0.49	5.58 ± 0.13
AEL	71.80 ± 0.79	44.16 ± 0.60	4.01 ± 0.16
AER	48.20 ± 0.79	18.12 ± 0.54	5.83 ± 0.09

Determination of secondary metabolites

Flavonoid content

MEL and AEL were the richest in flavonoids with levels of 59.97 ± 0.21 mg CE/g and 44.16 ± 0.60 mg CE/g respectively Table 1. However, the MER remains high with 31.22 ± 0.49 mg CE/g whereas the AER was only 18.12 ± 0.54 mg CE/g. Flavonoids represent about 68% of the leaf polyphenols. According to Marin et al.; Nazemiyeh et al., and El-Ansari et al., [30-32] the genus Salvia was rich in flavonoids. These were widely distributed in species of this genus [33,34].

MEL: Methanolic extract of leaves; MER: Methanolic extract of roots; AEL: Aqueous extract of leaves; AER: Aqueous extract of roots. The values represent the mean ± SD (n=3). [a]Milligrams gallic acid per gram of dry matter; [b]Milligrams catechin per gram of dry matter.

Content of condensed tannins

A preliminary screening showed the importance of this fraction compared to that hydrolysable. The results were reported in Table 1. AER and MER contain significant amounts of tannins, which were respectively 5.83 ± 0.09 and 5.58 ± 0.13 mg EC/g, somewhat greater than those of AEL and MEL with 4.01 ± 0.16 and 3.70 ± 0.09 mg CE/g ($P<0.05$), respectively. There were no previous studies that have targeted tannins in this species. The work done by Mahmoudi et al., [35], on *Cynara scolymus* L. shows that decoction is more efficient for tannin extraction (3.05 mg CE/g on average) than maceration (2.35 mg CE/g on average). The increase in temperature favors, on the one hand, the diffusion and solubility of the extracted substances and on the other hand, destroys certain fragile substances [36].

Antioxidant activity

The DPPH radical was generally one of the most widely used substrates for the rapid and direct evaluation of antioxidant activity due to its radical stability and the simplicity of the analysis [37]. The anti-radical activity profile of each extract, tested against the DPPH radical, is shown in Figure 2.

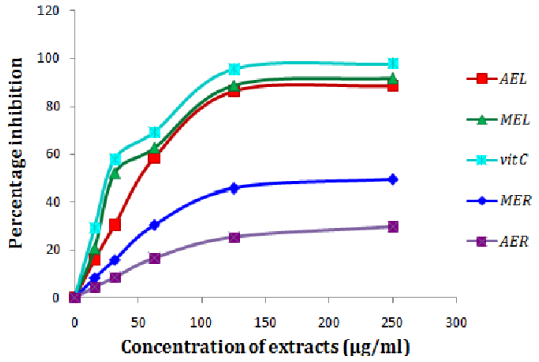

Figure 2: Anti-radical potency of *Salvia argentea* extracts

MEL: Methanolic extract of leaves; MER: Methanolic extract of roots; AEL: Aqueous extract of leaves; AER: Aqueous extract of roots; vit C: Vitamine C. The values used are mean ± standard deviation (n=3).

The level of DPPH reduced by the various antioxidants, measured at 517 nm, shows that the strongest anti-radical activities concern the methanolic (91.83 ± 0,74%) and aqueous (88.59 ± 0,46%) extracts of the leaves, while the lowest activities (49.29 ± 0,88% and 29.64 ± 0,41 %) were observed respectively for the methanolic and aqueous extracts of the roots at a concentration of 250 µg/ml. These results reveal that *S. argentea* has good antiradical activity particularly for MEL, which has a median inhibition concentration (IC50) of 45.42 ± 0.46 µg/ml. This value was significant compared with other leaf and root extracts Table 2. According to Lafka et al., [38], the antiradical activity can be affected by solvents of different polarity. Also, the antioxidant capacity of polyphenols depends mainly on the content of flavonoids [39].

Table 2: Median inhibition concentration of extracts of leaves and roots of *Salvia argentea*

Extracts	MEL	AEL	MER	AER	Vit C
IC_{50} (µg/ml)	45.42 ± 0.46	76.65 ± 0.44	216.89 ± 0.20	416.12 ± 0.26	16.97 ± 0.54

Values are presented as mean ± SD (n=3) (Figure 2)

The antioxidant activity of the various extracts makes it possible to classify them and show the effectiveness of the solvent in extracting the most antioxidant active ingredients as follows: MEL>AEL>MER>AER.

In view of these different results, the considerable polyphenolic content and the high flavonoid content of *S. argentea* may explain its high free radical scavenging activity due to its oxidation-reduction properties [40].

Iron reducing (FRAP)

The evaluation of the antioxidant activity by reduction of iron is an easy and reproducible method, for this it is very used to discern the most active extracts [41]. For all extracts, cascade dilutions ranging from 1 to 0.0625 mg/ml were prepared and the reducing

powers were measured at 700 nm. According to our results Figure 3, an increase in the absorbance corresponds to an increase in the reducing power of the extracts of *S. argentea*.

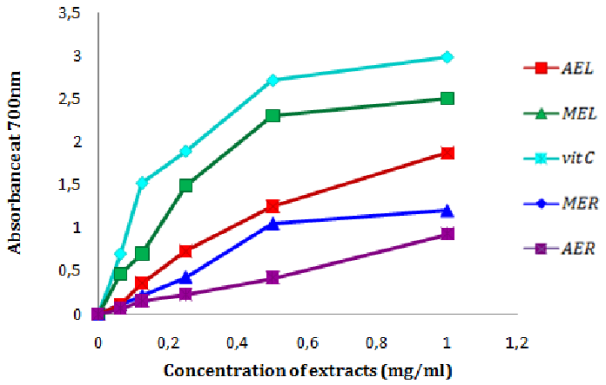

Figure 3: Anti-radical activity of the methanolic and aqueous extracts of the leaves and roots of *Salvia argentea*. The values used are mean ± standard deviation (n=3)

CONCLUSION

This work has for the first time concerned the determination of the primary and secondary metabolites of silver sage, a lamiaceae, and native of Algeria. Our results give a composition of the primary principles and show that secondary metabolites, mainly leaves, are of definite interest in medical therapeutics. Indeed, the important anti-radical activities of the aerial parts of this plant corroborate the numerous therapeutic virtues conferred on this type of plant since the Middle Age. It remains to value this plant by developing its nutraceutical potential and design for example a functional food containing its principles, with health effect.

REFERENCES

[1] F. Bourgaud, A. Gravot, S. Milesi, E. Gontier, *Plant Sci.*, **2001**, 161, 839-851.

[2] G.P.P. Kamatou, A.M. Viljoen, P. Steenkamp, *Food Chem.*, **2009**, 119, 684-688.

[3] M. Couladis, O. Tzakou, D. Stojanovic, N. Mimica-Dukic, R. Jancic, *Flavour and Fragrance Journal.*, **2001**, 16, 227-229.

[4] M. Tosun, S. Ercisli, M. Sengul, H. Ozer, T. Polat, E. Ozturk, *Biol Res.*, **2009**, 42, 175-181.

[5] M.H. Mirjalili, S. Peyman, A.Sonboli, M.M. Vala, *Chemistry of Natural Compounds.*, **2006**, 42, 1, 19-23.

[6] P. Quézel, S. Santa, Nouvelle flore de l'Algérie et des régions désertiques méridionales, Paris, 1963, Ed CNRS, 793.

[7] H. Lakhal, A. Kabouche, A. Alabdul Magid, L. Voutquenne-Nazabadioko, D. Harakat, Z. Kabouche, *PhytochemistrY.*, **2014**, 102, 145-51.

[8] L. Riccobono, A. Maggio, S. Rosselli, V. Ilardi, F. Senatore, M. Bruno, *Natural Product Research.*, **2015**, 30, 1, 1-10.

[9] M.F. Wang, J. Li, M. Rangarajan, Y. Shao, E.J. LaVoie, C.T. Ho, T.C. Huang, *J. Agr. Food. Chem. Y.*, **1998**, 46, 4869-4873.

[10] M. Kosar, H.J.D. Dorman, R. Hiltunen, *Food Chem.*, **2005**, 91, 525-533.

[11] F. Bekkara, M. Jay, M.R. Viricel, *J. Plant. Soil.*, **1998**, 203, 27-36.

[12] C. Audigie, J. Figarella, F. Zonszaain, Manipulation d'analyse biochimique, Paris, 1984, Ed Doin, 274.

[13] AOAC, Official methods of analyses, 1990, Washington, DC.

[14] ISO, Oil seeds, Determination of oil content, 2014, 659.

[15] J.D. Fox, J.F. Robyt, *Anal. Biochem.*, **1991**, 195, 93-96.

[16] S. Englard, S. Seifter, *Method. Enzymol*., **1990**, 182, 285-300.

[17] O.H. Lowry, N.J. Rosebrough, R. Randall, *J. Biol. Chem.*, **1951**, 193, 265-275.

[18] S.Y. Qusti, A.N. Abo-khatwa, M.A. Bin Lahwa, *EJBS.*, **2010**, 2, 1, 1-12.

[19] J.Zhishen, T. Mengcheng, W. Jianming, *Food Chem.*, **1999**, 64, 555-559.

[20] R. Julkunen-Tiitto, *Agric Food Chem.*, **1985**, 33, 2, 213-217.

[21] N. Benhammou, F. Bekkara, T. Kadifkova Panovska, C. R. Chimie., **2009**, 12, 1259-1266.

[22] M. Oyaizu, *Japn. J. Nutri.*, **1986**, 44, 307-315.

[23] R. Paris, M. Moyse, Précis de matière médicale, Paris, 1965, Ed. Masson., 412.

[24] M. Ben Farhat, A. Landoulsia, R. Chaouch-Hamadaa, J.A. Sotomayorc, M.J. Jordan, *Industrial Crops and Products.*, **2013**, 49, 904-914.

[25] M. Ben Farhat, A. Landoulsia, R. Chaouch-Hamadaa, J.A. Sotomayorc, M.J. Jordan, *Industrial Crops and Products.*, **2013**, 47, 106-112.

[26] A.V.P. Cuceu, M. Tofană, S. Socaci, M. Nagy, M.D. Borş, L. Salanţă, R. Vlaic, Bulletin UASVM Food Science and Technology., 2015, 72, 6-90.

[27] A. Lamien-Meda, M. Nell, U. Lohwasser, A. Borner, C. Franz, J.Novak, *J. Agric. Food Chem.*, **2010**, 58, 3813-3819.

[28] F. Shahidi, M. Naczk,. Food phenolics, sources, chemistry, effects, applications, 1995, Lancaster, PA: Technomic Publishing Co Inc, 1995.

[29] K.W. Lee, Y.J. Kim, H.J. Lee, C.Y. Lee, Food Chem., **2003**, 51, 7292-7295.

[30] P.D. Marin, R.J. Grayer, S. Grujic-Jovanovic, G.C. Kite, N.C. Veitch, *Phytochemistry.*, **2004**, 65, 9, 1247-1253.

[31] H. Nazemiyeh, M. Shoeb, N. Movahhedin, Y. Kumarasamy, A.H. Talebpour, A. Delazar, L. Nahar, S.D. Sarker, *Biochemlic Systematic and Ecology*. **2006**, 34, 721-723.

[32] M.A. El-Ansari, D. Barron, M.F. Abdalla, N.A.M. Saleh, *Phytochemistry.*, **1991**, 30,1169-1173.

[33] T. Adzet, S. Canigueral, J. Iglesias, *Biochemical Systematics and Ecology.*, **1987**, 16, 29-32.

[34] A. Ulubelen, E. Tuzlaci, *Fitoterapia.*, **1990**, 61, 185.

[35] S. Mahmoudi, M. Khali, N. Mahmoudi, *Nature and Technologie.*, **2013**, 9, 35-40.

[36] S. Jokić, D. Velić, M. Bilić, A. Bucić-Kojić, M. Plan inić, S.Tomas, *J. Food Sci.*, **2010**, 28, 206-212.

[37] M. Antolovich, P.D. Prenzler, E. Patsalides, S. McDonald, K. Robards, *Analyst.*, **2002**, 127, 183-198.

[38] T.I. Lafka, A.E. Lazou, V.J. Sinanoglou, E.S. Lazos, *Food.*, **2013**, 2, 18-31.

[39] H. Fenglin, L. Ruili, H. Bao, M. Liang, *Fitoterapia.*, **2004**, 75, 14.

[40] C.A. Rice-Evans, N.J. Miller, G. Paganga, *Trend. Plant. Sci.*, **1995**, 4, 152-159.

[41] H.B. Li, K.W. Cheng, C.C. Wong, K.W Fan, F. Chen, Y. Jiang, *Food. Chem.*, **2007**, 102, 771-776.