Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir

Comparative evaluation of different extraction methods for the assay of phytochemicals and antioxidant activity of *Valeriana officinalis* roots

Elnaz Hosseini Nia[1], Simin Arabi[*2], Malak Hekmati[1]

[1]*Department of Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran-Iran;*

[2]*Department of Chemistry, Safadasht Branch, Islamic Azad University,Tehran, Iran;*

Email:** ***siminarabi1354@yahoo.com

[3]*Department of Organic Chemistry, Faculty of Pharmaceutical Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran-Iran;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *April 29th 2020*
Accepted *July 26th 2020*

Key words:
- ✓ *Valeriana*
- ✓ *extraction method*
- ✓ *antioxidant*
- ✓ *total phenolic*
- ✓ *total flavonoids*

ABSTRACT

Background & Aim: Medicinal plants are rich sources of substances with nutraceutical and health benefits. *Valeriana officinalis* L. (*Valerianaceae*) is a known medicinal plant used in traditional medicine throughout the world. The present study aimed to evaluate the efficiencies of three methods for antioxidant extraction from *Valeriana officinalis*, and the impact of extraction methods on total phenollic and flavonoids contents and antioxidant activities of valeriana root was studied.

Experimental: The dried-root was extracted by three different methods including maceration, ultrasonic assisted, and Soxhlet assisted extraction. Antioxidant capacity of the extracts was assessed using DPPH and nitric oxide (NO) free radicals scavenging, reducing power and iron chelating activity. Total phenolic and flavonoid contents were also identified.

Results: The ultrasonic extract showed the highest amount of total phenolics and flavonoids contents. In DPPH radical scavenging activity and reducing power assay, the ultrasonic assisted extract, (IC_{50}=54.6 µg/ml) showed higher activity than the other extracts. In DPPH radical scavenging activity, IC_{50} for ultrasonic extract, soxhlet assisted extraction and maceration extract were 54.6, 81.6 and 67.8 µg/ml, respectively. The results clearly showed that the extraction methods used in this study significantly affected antioxidant capacities and total phenolic and flavonoids contents. It was found that ultrasonic assisted extraction and Soxhlet methods are more efficient in extraction of antioxidant components from *Valeriana officinalis* L.

Recommended applications/industries: Considering result of study, it can be inferred that the herb may be a good source of bioactive compounds and can work as an antioxidant to prevent the oxidative deteriorative activity of food materials.

1. Introduction

The roles of oxidants have been identified in many acute and chronic diseases including cancer, cardio vascular problems and neurodegenerative diseases. Medicinal plants are consisted of a wide variety of natural antioxidant, such as phenolic acids, flavonoids and tannins, which have more antioxidant property than dietary plants (Kaur and Kapoor, 2002). Recently, more attention has been paid to medicinal plants for their phenolic compounds and related potent antioxidant activity. The investigations have shown that natural antioxidants have a great value in prevention or treatment of pathological disorders (Chang *et al.*, 2002). The genus *Valeriana officinalis* belongs to *Valerianaceae*, which is spread throughout the world. *Valerian* species (*Valerianaceae*) have numerous medicinal applications as stone inhibitor, expectorant, anti-inflammatory and sedative properties, also antioxidant activities of this plant's root have recently been reported (Balasundram *et al.*, 2006). In this study, we evaluated the efficiencies of three methods (ultrasonically assisted extraction, Soxhlet extraction and maceration method) for extracting antioxidants from *V. officinalis*. The antioxidant capacity was assessed using four different methods including 1,1-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) free radical scavenging, reducing power and iron chelating activity. The total phenolic and flovonoids contents in different extracts were also studied. This study tries to determine a scientific basis application of this plant in traditional medicine, and to find the possible mechanism involved in its antioxidant activity.

2. Materials and Methods

2.1. Plant material and preparation of extracts

Roots of *V. officinalis* were collected from Lorestan, Iran. Then, the roots were dried in shade at room temperature and then were grounded in a course powder with a suitable grinder. The powder was stored in an airtight container under cool and dry place until further analysis. For extract preparation (a) 50 g of dried powder was macerated for 24h with 300 ml of methanol. Extraction was repeated three times. The extracts were filtered through Whatman filter paper No.1, and the resultant extracts were evaporated using rotary evaporator until a solid sample was obtained (maceration extracts); (b) The sample was extracted exhaustively in a Soxhlet extractor with methanol for 24h. The crude solid extracts were evaporated for complete solvent removal and used as Soxhlet extracts; (c) The sample was extracted with 300 ml methanol in an ultrasonic bath at a frequency of 100 kHz and temperature of 25°C for one hour (3×20 min) to yield ultrasonic extracts (Motallebi Riekandeh *et al.*, 2016). The extract was filtered by Whatman filter paper No.1, and the filtrate was concentrated under reduced pressure at 40°C. The extract (2.4 g) was kept at 4°C in vials and applied as ultrasonic extract (Ma *et al.*, 2008).

2.2. Total Phenolic Content

The total phenolic contents of the extract were evaluated by Folin–Ciocalteu method (Prior *et al.*, 2005), after that the extract samples (0.5 ml of different dilutions from 25-800 µg $ml^{-1}$of extract) were mixed with 2.5 ml of 0.2N Folin-Ciocalteu reagents for 5 min and then by 2 ml of 20% (w/v) sodium carbonate was added. The mixture was kept for a further 2 hour in the dark, and absorbance of reaction was measured at 760 nm. The standard curve was drawn by 20-100 µg ml^{-1} solution of gallic acid. The total phenolic content was calculated from the *calibration* curve, and the results were stated as mg of gallic acid equivalent per g dry weight.

2.3. Total Flavonoid Content

The total flavonoid contents were measured by the aluminum chloride colorimetric method (Kim *et al.*, 2003). Briefly, 0.5 ml of extracts in the concentration of 25-800 µg ml^{-1} were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum Chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The mixture was kept for 15 min, and absorbance of the reaction mixture was measured at 415 nm. Standard curve was prepared by quercetin at concentrations 10-100 µg ml^{-1}. The total flavonoid contents were computed in terms of quercetin equivalents (QE) from a calibration curve, and the result was expressed as mg quercetin equivalents (QE) per g dry weight.

2.4. Antioxidant properties

2.4.1. DPPH radical-scavenging activity

Two milliliter of different concentrations of each extracts (25-800 μg ml^{-1}) were mixed with 2 ml methanolic solution of DPPH (100 μM). After 15 minutes at room temperature in the dark, the absorbance was recorded at 517 nm. BHT was used as standard controls. IC_{50} values indicate the concentration of sample, which is needed to scavenge 50% of DPPH free radicals (Bondet *et al*., 1997).

2.4.2. Iron chelating capacity

Different concentrations of extracts (25-800 μg ml^{-1}) were added to a solution of 0.05 ml of ferrous Chloride (2mM). 0.2 ml of ferrozine solution was added to examine the strong iron chelating properties of the extracts. After 10 minutes, the absorbance of the solution was recorded at 562 nm. EDTA was used as a standard (Huang *et al.*, 2005).

2.4.3. Ferric reducing power assay

The reducing powers of extract were evaluated according the method of Yen and Chen (1995). Fe (II) reduction is often used as an indicator of electron donating potential, which is a significant mechanism of phenolic antioxidant activity (Jayaprakash *et al.*, 2001). Briefly, different amounts of extracts (25-800 μg ml^{-1}) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min, and a portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction. The mixture was centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Ascorbic acid was used as positive control.

2.4.4. Nitric oxide-scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. It is known that sodium nitroprusside is decomposed in aqueous solution at physiological pH (7.2) spontaneously and produced nitric oxide (NO), which interacts with oxygen to induce nitrite ions that can be determined using Greiss reagent. Scavengers of nitric oxide compete with oxygen which leads to reduction of nitric oxide production. For the experiment, 1 ml of sodium nitroprusside (10 mM) in phosphate-buffered saline was mixed with different concentrations of methanolic extracts (5–200 μg/ml) and incubated at 30°C for 2h. The same reaction mixture without the extract and the equivalent amount of methanol was used as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore, which was formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride, was immediately read at 546 nm. Quercetin was used as positive control (Hoya *et al*., 2010).

2.5. Statistical analysis

All experiments were performed with three replication and the results were expressed as mean ± SD. The data were subjected to the analysis of variance (ANOVA) and Duncan's multiple range tests was used to compare means at a significant level of 5% (P<0.05). To calculate IC_{50} for each plant extract, linear regression analysis was used.

3. Results and discussion

3.1. Total phenolic and flavonoid contents

Natural phenolic compounds act as reducing agents and quenchers of singlet oxygen, so some of them can play an important role in cancer prevention and treatment (Cai *et al*., 2004). Investigations have shown that consumption of foods and beverages rich in phenolic content is related to the reduction of atherosclerosis and cardiovascular diseases risk (Kasote *et al*., 2015). Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds induce their chemopreventive properties such as antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects and also regulating carcinogen metabolism and ontogenesis expression by arresting cell cycle, inhibiting DNA binding and cell adhesion,

migration, proliferation or differentiation, and blocking signaling pathways contribute to their inducing apoptosis (Saeed *et al*., 2012). The total phenolic contents of the three methods (ultrasonically assisted extraction, Soxhlet extraction and maceration method) were estimated by Folin Ciocalteu's method using gallic acid as standard. The gallic acid solution of the concentration (20-100 ppm) conformed to Beer's Law at 760 nm with a regression co-efficient R^2 = 0.9941. Total phenolic contents of *V. officinalis* root were between 312.3-390.6 mg gallic acid equivalent (GAE)/g of extract (Table 1). The total phenolic contents were in order of: Ultrasonic extract > Soxhlet extract > Maceration extract, respectively. This is due to the acoustic cavitation phenomena, which will lead to high shear forces and turbulences in the solvent. When the ultrasonic wave propagates into the solvent, formation, growth and implosion of bubbles occur. The implosion of the bubbles on the surface of the plant material creates high-speed jets of solvent into the surface and causes shock wave damage. This can lead to erosion and fragmentation of plant material, hence increasing the mass transfer rate. For instance, Chemat *et al*. (2017) reported that the oil gland at the surface of basil leaves ruptured after exposure to 25 kHz sonication, whereas the oil gland still remain intact when treated by maceration. They attributed the oil gland damage to the shear forces generated at the implosion of bubbles near the oil gland (Chemat *et al*., 2017).

	Extraction methods		
	Maceration	**Soxhlet**	**Ultrasonic**
Total phenolic contents (GAE mg/g)	312.3±3.2^c	342.7±2.7^b	390.6±1.3^a
Total flavonoid contents (QE mg/g)	79.6±1.5^c	102.4±1.7^b	127.3±2.4^a

Table 1. Total phenolic and total flavonoid contents in *V.officinalis* root extracts.
Data were presented as Mean ± SD. Different letters in each row indicate a significant difference ($p<0.05$).

Flavonoids have been linked to reduction of the risk of major chronic diseases including cancer because they have powerful antioxidant activities in vitro, being able to scavenge a wide range of reactive species (e.g., hydroxyl radicals, peroxyl radicals, hypochlorous acid, and superoxide radicals) (Butkovic *et al*., 2004). Many of flavonoids chelate transition metal ions such as iron and copper and decrease their ability to promote reactive species formation. These activities of flavonoids are associated with their structures. Flavonols which contain more hydroxyl groups exhibit very high radical scavenging activity, for example, myricetin, quercetin, rutin, and quercitrin are well known potent antioxidants. In Flavanols with additional catechol structure (3-galloyl group) antiradical activity has significantly increased (Kawada *et al*., 1998). Moreover, the antioxidant activity of flavonoids is affected by glycosylation of hydroxyl groups and substitution of other components (e.g., methoxy groups). The total flavonoid content of three methods (ultrasonically assisted extraction, Soxhlet extraction and maceration method) was measured with the aluminum chloride colorimetric assay using quercetin as standard. Aluminium chloride forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols, which gives maximum absorption at 510 nm (Kerry and Abbey, 1998). The quercetin solution of concentration (10-100 ppm) conformed to Beer's Law at 415 nm with a regression co-efficient R^2 = 0.9996. The total flavonoid contents of extracts were between 79.6-127.3 mg quercetin equivalent (QE) /g of extract, as derived from a standard curve (Table 1). The total flavonoid contents were in order of: Ultrasonic extract > Soxhlet extract > Maceration extract, respectively. Ultrasonic extract had also higher flavonoids contents comparing other extracts. Data obtained from the total phenolic content and total flavonoid content and methods confirm the key role of phenolic compounds in free radical scavenging and/or reducing systems. Antioxidant activity of *V. officinalis* is a result of phenolic acid, especially caffeic, *p*-coumaric and ferulic acid content. Caffeic acid is expected to have higher antioxidant activity because of additional conjugation in the propenoic side chain, which might facilitate the electron delocalization, by resonance, between the aromatic ring and propenoic group. Other studies have also reported that *V. officinalis* was rich in phenolic constituents and showed good anitioxidant activities (Wojdyło *et al*., 2007).

3.2. DPPH radical-scavenging activity

Plants rich in secondary metabolites, including phenolics, flavonoids and carotenoids, have antioxidant activity due to their redox properties and chemical structures. The methanolic root extract of *V. officinalis* had strong antioxidant activity against all the free radicals studied. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Stasko *et al*., 2007). Phenols are very important plant constituents because of their scavenging ability on free radicals due to having hydroxyl groups. Therefore, the phenolic compounds in plants may contribute directly to their antioxidant action (Tosan *et al*., 2009). In the DPPH assay, the antioxidants are able to donate hydrogen for reduction of the stable radical DPPH to the yellow-colored non-radical diphenyl- picrylhydrazine (DPPH-H). The capacities of extracts to scavenge DPPH are shown in Table 2. Ultrasonic extract showed the highest activity (IC_{50} = 54.6 ± 0.53 µg ml^{-1}) followed by Soxhlet extract with IC_{50} = 67.8 ± 0.26 µg ml^{-1}. BHA was used as positive control and IC_{50} of BHA was 52.9 ± 0.32 µg ml^{-1}. All extracts showed a concentration-dependent antiradical activity by inhibiting DPPH radical. Ultrasonic extract with higher phenol and flavonoids contents showed the best activity and maceration extract with the lowest phenol and flavonoid contents were the least active.

	Extraction Methods			Control		
	Ultrasonic	Soxhlet	Maceration	BHA	EDTA	Quercetin
DPPH radical scavenging IC_{50} (µg/ml)[a]	54.6± 0.53[c]	67.8±0.26[b]	81.6±0.42[a]	52.9±3.2[c]	---	---
Fe^{2+} chelating ability IC_{50} (µg/ml)[b]	186.3±5.2[c]	202.2±8.3[b]	421.3±14.2[a]	---	16.3±0.3[d]	---
NO scavenging activity IC_{50} (µg/ml)[c]	59.1±0.03[b]	76.4±0.04[a]	16.9±0.6[c]	---	---	19.0±0.02[c]

Table 2. Antioxidant activities of *V. officinalis* root extracts.
Data were presented as Mean ± SD. Different letters in each row indicate a significant difference (P<0.05).

3.3. Reducing power

Antioxidant compounds reduce Fe^{3+}-ferricyanide complexes to the ferrous (Fe^{2+}) form. By adding $FeCl_3$, the ferric form (Fe^{3+}) converted to the ferrous form (Fe^{2+}) and the Prussian blue colored complex is formed. Therefore, the reduction amount can be determined by measuring the formation of Perl's Prussian blue at 700 nm. In this assay, depending on the reducing power of the antioxidant the yellow color of the test solution changes to green or blue. A higher absorbance shows higher ferric reducing power (Chung *et al*., 2002). Figure 1 shows the dose-response curves for the reducing powers of the extracts. The reducing power of the extracts increased with the increase in their concentrations. Increasing the absorbance at this wave length indicates an increase in the reducing power. The extracts exhibited fairly good reducing power at concentration of 25 and 800 µg ml^{-1}, although the values were less than that of vitamin C as positive control (P<0.001).The ultrasonic extract showed better activity than vitamin C. Because of the good reductive ability of ultrasonic extract, it was evident that this extract showed reductive potential and could act as strong electron donors and terminate the radical chain reaction.

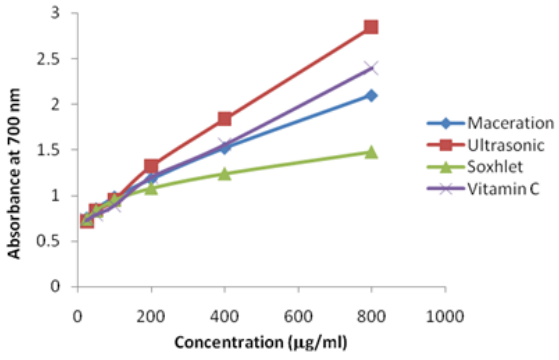

Fig 1. Reducing power of *V. officinalis* root extracts by different extraction methods.

3.4. Nitric oxide-scavenging activity

Nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various

physiological processes. However, excessive production of NO is related to several diseases. Nitric oxide is a very unstable species under aerobic conditions. It reacts with O_2 to produce stable product nitrate and nitrite through intermediates NO_2, N_2O_4, and N_3O_4. NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. Using Griess reagent and in presence of the extracts, it is estimated that the amount of nitrous acid will decrease (Shirwaikar *et al.,* 2006). The relation of nitric oxide (NO) with cardiovascular disease has long been recognized and the extensive research on this topic has revealed both pro- and anti-atherosclerotic effects (Alisi and Onyeze, 2008). The results of NO scavenging activity of extracts are indicated in Table 2. Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 25°C for 2h resulted in linear time-dependent nitrite production, which is decreased by the tested methanolic extracts of *Valeriana officinalis.* This may be due to the antioxidant properties in the extract, which compete with oxygen to react with nitric oxide and thereby inhibits the generation of nitrite. It should be noted that ultrasonic extract showed a greater inhibition activity compared to others but less activity than quercetin. Nitric oxide scavenging activity of the extracts was between 16.9 and 76.4 mg ml^{-1} (Table 2). Inhibition increased by increasing the concentration of the extracts. The activity was in order of Ultrasonic extract > Soxhlet extract > Maceration extract. Phenolic compounds and flavonoids have been reported to be involved in the antioxidative action in biological systems, and act as scavengers of singlet oxygen and free radicals (Rice-Evans *et al*., 1997). We can consider that these constituents might be responsible for the observed nitric oxide scavenging activity, since the nitric oxide scavenging activity of flavonoids and phenolic compounds are well known (Wink *et al*., 1991).

3.5. Iron Chelating Capacity

Ferrozine can quantitatively form complexes with Fe^{2+}. The absorbance of Fe^{2+}-ferrozine complex dose-dependently decreased. Ultrasonic extract showed the best activity followed by Soxhlet extract. Ultrasonic extract had the highest amount of phenol and flavonoid contents (Table 2). Flavonoids with a certain structure and particularly hydroxyl position in the molecule are known to serve as proton donator and show radical scavenging activity (Firuzi *et al*., 2005).

4. Conclusion

The extraction efficiency of the three methods (ultrasonic assisted extraction, Soxhlet extraction and maceration method) for the extraction of antioxidant compounds from *V. officinalis* root was evaluated. The results clearly indicated that the extraction methods significantly affect antioxidant capacities and total phenolic and flavonoids contents of the extracts. It was found out that Ultrasonic assisted extraction and Soxhlet methods are more efficient in extraction of antioxidant components of *V. officinalis* than the maceration method.

5. Acknowledgements

The authors gratefully acknowledge Islamic Azad University, Pharmaceutical Sciences Branch, for support.

6. References

Alisi, C.S. and Onyeze, G.O.C. 2008. Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.). *African Journal of Biochemistry Research*, 2(7): 145-150.

Balasundram, N., Sundram, K. and Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1): 191–203.

Bondet, V., Brand-Williams, W. and Berset, C. 1997. Kinetics and Mechanisms of Antioxidant Activity using the DPPH. Free Radical Method. *LWT-Food Science and Technology*, 30(6): 609–615.

Butkovic, V., Klasinc, L. and Bors, W. 2004. Kinetic study of flavonoid reactions with stable radicals. *Journal of Agricultural and Food Chemistry*, 52(10): 2816–2820.

Cai, Y., Luo, Q., Sun, M. and Corke, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, 74(17): 2157–2184.

Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of total flavonoid content in propolis by

two complementary colorimetric methods, *Journal of Food* and *Drug Analysis*, 10(3):178–182.

Chemat, F., Rombaut, N., Sicaire, A.G, Meullemiestre, A.S.F. Tixier and Vian, M.A. 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, 34: 540-560.

Chung, Y-C., Chang, C-T., Chao, W-W., Lin, C-F. and Chou, S-T. 2002. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. *Journal of Agricultural and Food Chemistry*, 50(8): 2454–2458.

Firuzi, O., Lacanna, A., Petrucci, R., Marrosu, G. and Saso, L. 2005. Evaluation of the antioxidant activity of flavonoids by ferric reducing antioxidant power assay and cyclic voltammetry. *Biochimica et Biophysica Acta*, 1721(1-3): 174-184.

Hoya, L., Biendl, M. and Heyerick, A. 2010. Radical Scavenging Capacity of hop-derived product. *Brewing Science*, 63(1): 1-5.

Huang, D., Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6): 1841–1856.

Jayaprakash, G.K., Singh, R.P. and Sakariah, K.K. 2001. Antioxidant activity of grape seed extracts on per oxidation models in vitro. *Journal* of *Agriculture* and *Food Chemistry*, 55: 1018-1022.

Kasote, D.M., Katyare, S.S., Hegde, M.V. and Bae, H. 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, 11(8): 982–991.

Kaur, C. and Kapoor, HC. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal* of *Food Science* & *Technology*, 37(2): 153–161.

Kawada, N., Seki, S. and Kuroki, T. 1998. Effect of antioxidants resveratrol, quercetin and N-acetylcystein, on the functions of cultured rat hepatic stellate cells and kupfer cells. *Hepatology*, 27(5): 1265–1274.

Kerry, N. and Abbey, M. 1998. The isoflavone genistein inhibits copper and peroxyl radical mediated low density lipoprotein oxidation in vitro. *Atherosclerosis*, 140(2): 341–347.

Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y. and Lee, C.Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry,* 51(22): 6509–6515.

Ma, Y., Ye, X., Hao, Y., Xu, G. and Liu, D. 2008. Ultrasound-assisted extraction of hesperidin from Penggan (Citrus reticulata) peel. *Ultrasonics Sonochemistry*, 15(3): 227-232.

Motallebi Riekandeh, S., Mazandarani, M., Ebrahimzadeh, M.A. and Zargari, M. 2016. Antioxidants activities of Eryngium caucasicum inflorescence. *European Review for Medical and Pharmacological Sciences*, 20(5): 946-949.

Prior, R., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10): 4290–302.

Rice-Evans C., Sampson, J., Bramley, P.M. and Holloway, D.E. 1997. Why do we expect carotenoids to be antioxidants in vivo. *Free Radical Research*, 26(4): 381-398.

Saeed, N., Khan, M.R. and Shabbir, M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complementary and Alternative Medicine*, 12: 221-232.

Shirwaikar, A., Prabhu, K.S. and Punitha, I.S.R. 2006. In vitro antioxidant studies of Sphaeranthus indicus (Linn), *Indian Journal of Experimental Biology*, 44(12): 993–996.

Stasko, A., Brezová, V., Biskupic, S. and Misík, V. 2007. The potential pitfalls of using 1,1- diphenyl-2 picrylhydrazyl to characterize antioxidants in mixed water solvents. *Free Radical Research*, 41(4): 379–390.

Tosun, M., Ercisli, S., Sengul, M., Ozer, H., Polat, T. 2009. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biological Research*, 41: 175-181.

Wink, O.A., Kasprzak, K.S. and Maragos, C.M. 1991. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science*, 254(5034): 1001-1003.

Wojdyło, A., Oszmianski, J. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105: 940-949.