

Composition and antioxidant activities of Iranian *Pulicaria gnaphalodes* essential oil in Soybean oil

Nabi Shariatifar¹, Abolfazl Kamkar², Mohammad Reza Shamse Ardekani³, Ali Misagi⁴, Afshin Akhonzade⁴ and Amir Hossein Jamshidi⁵

¹Department of Environment Health, School of Public Health, Tehran University of Medical Sciences (TUMS), Tehran, Iran

²Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

³Department of Pharmacognosy, University of Medical science Tehran, Tehran, Iran

⁴Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

⁵Food and Drug Deputy, Ministry of Health and Medical Education, Iran

Abstract: The essential oil from aerial parts of *Pulicaria gnaphalodes* was studied in soybean oil. The aim of this study was to evaluate the antioxidant activities of Iranian *Pulicaria gnaphalodes* essential oil in soybean oil during the storage period. The essential oil obtained from *Pulicaria gnaphalodes* by hydrodistillation and analyzed by GC/Mass. Fifty-eight compounds representing 90.7% of total was identified. Main ingredient in the oil were involved α -Pinene (30.2%), 1,8-Cineole (12.1%), Beta-Citronellol (9.6%), Mertenol (6.6%), α -Terpineol (6.1%), 4-Terpineol (5.9%) and Chrysanthenone (2.9%). Different concentrations (0.200, 400 and 800 ppm) of essential oil and β hydroxyl toluene (BHT; 100 and 200 ppm) was added to soybean oil and incubated for 35 days at 65°C. Peroxide values (PVs) and thiobarbituric acid-reactive substances (TBARs) levels were measured every week during the time period of the study. Moreover, antioxidant capacity of the essential oil was determined using 1,1 diphenyl-2-picryl hydrazyl (DPPH) and β -carotene-linoleic acid methods. Values were compared among groups in each incubation time using ANOVA test. Results revealed that DPPH β -carotene-linoleic acid assay findings on the *P. gnaphalodes* essential oil were lower than these of synthetic antioxidant, BHT. Moreover, during the incubation time, *P. gnaphalodes* essential oil lowered PVs and TBARs levels when compared to the control ($p < 0.001$). According to our results essential oil was less effective than synthetic antioxidant. Therefore it may be used as a food flavor, natural antioxidant and a preventive agent for many diseases caused by free radicals.

Keywords: Antioxidant activity, *Pulicaria gnaphalodes*, essential oil, Soybean oil.

INTRODUCTION

Some of the compounds found in plants have potent antioxidant activity. Antioxidants in food play an important role in human health. Antioxidant activity, one of the most important physiological roles of food is supposed to protect organisms from oxidative damage, and causes severe refractory disease prevention, including various types of cancer, coronary heart disease diabetes, neurological diseases and is and other disease (Ames *et al.*, 1993; Velioglu *et al.*, 1998; Wang *et al.*, 1996 and Dauchet *et al.*, 2005).

Many epidemiological studies have shown a link between consumption of plant derived food, such as fruits, vegetables and legumes and decrease risk of coronary heart diseases, it is thought that an increase consumption of plant derived food improve the health status and delay the development of chronic diseases in human. This thought, initiated numerous experimental studies dedicated to understanding the role of dietary antioxidants in the prevention of disease (Dauchet *et al.*, 2005; Hu., 2003).

In addition, foodstuff international organizations have

*Corresponding author: e-mail: nshariatifar@ut.ac.ir

new approach to health food and used natural food preservative instead of chemicals preservative. Because of the possible adverse effects of high doses of natural food preservative on taste, smell and color of food not met with great interest, but the rose plant is compatible with popular tastes.

Pulicaria is a plant genus in the family of *Asteraceae*. This species is generally known under the name “kak kosh” and “Shabang” in Iran and are commonly used as herbal tea, flavoring agent, and medicinal plant. *P. gnaphalodes* (Vent.) Boiss. is a persistent plant of about 10-30 cm high, with gold-yellow flowers, which grow on sandy, stony places in the Asia (Shariatifar, 2011). Kakkosh-Biabani (*Pulicaria gnaphalodes* Linn) is a plant which grows wild in abandoned areas such as Iran, Iraq, Pakistan, India, Turkey, Saudi Arabia (Mozaffarian, 1996). This plant is a worldwide known folk medicine for severe dehydrations, inflammation, etc (Shariatifar, 2011). The chemical investigation of the genus showed the presence of terpenes such as monoterpenes and oxygenated monoterpenes, diterpenes and sesquiterpenes (Eshbakova, 2011; Khani and Asghari, 2012).

This plant known background and because it grows in many Province's in Iran and as a result, we can say that

it's to be prepared very cheap. It may be a good source of natural antioxidants and a good food preservative to prevent spoilage of food and improve the quality of food products.

The aim of this study was to analyses the composition of the essential oil from Kakkosh-Biabani of Iran and evaluates antioxidant activity of essence in soybean oil as a food system.

MATERIALS AND METHODS

Sample and reagents

Aerial parts of *P.gnaphalodes* L. were collected from Khorasan Razavi province (Iran) during the flowering period in summer 2010. A voucher specimen for this plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Tehran University, Tehran, Iran. The plants were dried in a dark place at room temperature. Dried leaves were powdered using an electrical device (MJ176NR/Japan) and stored at 4°C until use. Soybean oil without any antioxidants was taken from the manufacturing company (Behahahr, oil Manufactory, Tehran, Iran).

Preparation of stripped soybean oil

The method described by Yoshida (1993) with some modifications, to remove the tocopherols from soybean oil by column chromatography using alumina. The column was rolled in aluminium foil to avoid oxidation. The soy bean oil was drawn through the column by suction.

The Soy bean oil collected was again passed through fresh alumina to complete removal of the tocopherols. Antioxidants (essential oil and BHT) were added to soybean oils free from tocopherols.

Extraction of the essential oil

In the present study, essential oil was extracted using the procedure of Kantar *et al.* (2003). Leaves of *Pulicaria gnaphalodes* (100g) were placed in round bottom flask (2L), water was added (1:6 v/v) and heated in Hydro Distillation Apparatus for 4 hours at 100°C. The essential oil on water surface was collected by decanting the water through the tap. The moisture content from the oil was removed with anhydrous sodium sulfate. Yield of essential oil was determined and stored at 4°C until further use (Kanter *et al.*, 2003; Rădulescu *et al.*, 2008).

GC/Mass analysis

The essential oil was analyzed by GC/Mass using Agilent 6890 mass selective detector technology connected to a HP 5975 gas Chromatograph. The separation was achieved by capillary column, HP-MSD (5% phenyl methyl poly siloxane) (30m×0.25mm, film thickness 0.20µm). The column temperature was kept at 50°C for 5

min and programmed to 265°C at a rate of 2.5°C/min. The flow rate of helium was 1.1ml/min and MS was taken at 70 ev. (electron volt). The relative amounts of the oil constituents were expressed as percentages by peak area normal. The identification of individual compound was based on comparison of their relative retention indices with those of authentic samples on the HP-MSD Capillary column and by matching of their mass spectra with those obtained from Wiley libraries and published data (Rădulescu *et al.*, 2008).

In vitro anti oxidant activity

DPPH assay

The hydrogen atom or electron donation abilities of the corresponding essential oil and some pure compounds were measured from the bleaching of the purple colored methanol solution of 2, 2-diphenyl 1-1-picrylhydrazyl (DPPH). This spectrophotometric assay was done using the stable radical DPPH as a reagent according to the method of Burits and Bucar (Burits *et al.*, 2000, Dasgupta *et al.*, 2013). First 50µL of the extracts (various concentrations) were added to 5ml of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radial scavenging activities of the samples were calculated as percentage of inhibition according to the following equation:

$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$, where A_{blank} is the absorbance of the control (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Essential oil concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition percentage against essential oil concentration. All tests were done in triplicate. Values (mean±SD) of the essential oil were compared with those of BHT using student's t- test. A peroxide value less than 0.05 was considered significant statistically.

B-Carotene-linoleic acid assay

Antioxidant capacity was determined by measuring the inhibition of volatile organic compounds and the conjugated diene -hydro peroxides obtained from linoleic acid oxidation according to the method of Dapkevicius *et al.*, (1998)(15). The stock solution of β-Carotene – linoleic acid mixture was prepared as follows; 0.5mg β-Carotene (Merck) was dissolved in 1 ml of chloroform (HPLC grade) and then 25µL linoleic acid (Sigma) and 200mg Tween 40 (Merck) were added. After the evaporation of chloroform, 100 ml of oxygen saturated distilled water was added with vigorous shaking. Then, 2500 µL aliquots were dispensed into the test tubes, 350 µL of the essential oil (2g/L) was added and the emulsion system incubated for 48 hour at room temperature. The same procedure was performed for both BHT (as positive control) and blank. In turn, absorbance spectra of the mixtures were obtained at 490 nm. Afterward, antioxidant capacities of the extracts were compared with those of

BHT and blank. Further, all inhibition percentages were compared using with 95% confident interval.

Antioxidant activities of the essential oil in soybean oil

Antioxidant activities of essential oil on lipid peroxidation were appraised in soybean oil according to method of Duh (1999) with some modifications. Each sample (50ml) was transferred to a series of capped glass test tubes. Then, *P. gnaphalodes* essential oil (0,200,400 and 800 ppm) and BHT (100,200) were added to the test tubes and put in a dark oven at 65°C. The stability of oil to oxidation was appraised each week over a 5- week period by analyzing the peroxide values (PVs) and TBARs levels (16).

Peroxide value

The peroxide value was measured by AOCS cd 8-53 official Method (1981). For this purpose, a known weight of oil sample (3g) was dissolved in glacial acetic acid (30ml) and chloroform (20ml). Then saturated KI solution (1ml) was added. The mixture was kept in the dark for 15 min. After adding distilled water (50ml), Mixture was titrated against sodium thiosulphate (0.02N) using starch as indicator. The Peroxide values (PVs) (meq of oxygen/kg) were calculated using the following formula: peroxide value = $1000S \times N/W$. In this formula, S is the volume of sodium thiosulphate solution (blank corrected) in ml; N is the normality of sodium thiosulphate solution (0.02) and W is the weight of oil sample (gram) (AOCS., 1981).

Table 1: Chemical composition of the essential leaf oil of *P. gnaphalodes* from Iran

S. No	Compound	*KI	Time (min)	Percentage
1	Alpha-thujene	931	13.58	0.2
2	Alpha-pinene	939	14.08	30.2
3	Camphene	953	14.89	0.1
4	Verbenen	967	15.19	0.1
5	Sabinene	976	16.26	0.1
6	pinene-Beta	979	16.54	0.5
7	Dehydro-1-8-cineole	982	17.28	0.1
8	1-Phellanderin	1005	18.37	0.2
9	Alpha--Terpinene	1018	18.88	0.9
10	P-cymene	1026	19.48	1.3
11	1-8-cineole	1033	20.07	12.1
12	Gamma-Terpinene	1058	21.5	1.4
13	Trans-sabinene-hydrate	1058	21.6	0.6
14	AlPha-Terpinolene	1087	23.3	0.5
15	Filifolone	1088	23.67	0.8
16	Linalool	1098	23.95	0.9
17	Chrysanthenone	1123	24.63	2.9
18	1-Terpineol	1141	26.58	0.7
19	Camphor	1149	26.93	0.3
20	Nerol oxid	1153	27.3	0.3

S. No	Compound	*KI	Time (min)	Percentage
21	Cis- Chrysanthenol	1157	28.09	0.4
22	4-Terpineol	1177	29.1	5.9
23	Alpha-Terpineol	1189	29.92	6.1
24	Mertenol	1194	30.38	6.6
25	Alpha-Campholenol	1196	30.38	0.1
26	Beta-Citronellol	1228	32.2	9.6
27	Neral	1237	32.77	0.7
28	Cis-Chrysanthenyl formate	1239	32.9	t
29	Alpha- Citral	1270	34.46	0.2
30	Thymol	1290	34.64	1.6
31	Geranyl formate	1283	34.39	t
32	Trans-Anethole	1284	35.4	t
33	Mertenyl acetate	1306	37.04	t
34	Citronellyl acetate	1336	35.65	0.1
35	Alpha-Cubebene	1352	36.05	t
36	Neryl acetate	1365	39.55	t
37	Eugenol methyl ether	1376	39.77	0.2
38	Beta-copaene	1378	40.58	t
39	Geranyl acetate	1383	40.69	t
40	Alpha-Gurjunene	1412	42.63	t
41	Beta -caryophyllene	1422	42.88	0.1
42	Neryl acetate	1434	44.42	t
43	Allo-Aromadendrene	1462	45.48	0.3
44	Beta-Ionone	1467	46.49	0.4
45	Beta-Selinene	1484	46.84	0.1
46	Curcumene	1485	46.95	0.1
47	Cis-Calamenene	1486	47.35	0.3
48	Alpha-Murolene	1499	48.06	0.3
49	Gamma-Cadinene	1519	48.56	0.6
50	Delta-Cadinene	1524	49.65	0.7
51	1,8-Epoxycadin-4-ene	1550	50.24	0.1
52	Alpha-Coppaen -8α-ol	1555	51.12	0.2
53	Caryophyllene epoxide	1564	51.92	0.2
54	Palustrol	1578	52.76	t
55	Humulene epoxide II	1598	52.97	0.1
56	Ledol	1598	53.73	0.3
57	Alpha-cadinol	1643	55.3	0.1
58	Cadalene	1662	62.24	1.1

Thiobarbitoric acid-reactive substances (TBARs)

Thiobarbitoric acid-reactive substances (TBARs) were determined weekly, using the method of AOCS (1998). This procedure allows the direct determination of TBARs in oils and fats without preliminary isolation of secondary oxidation products. Oil sample (50-200mg) was solubilized in 10ml of 1- butanol, mixed with 10ml of 0.2% TBA in 1- butanol, incubated 2hour in a 95C water bath and cooled for 10 min under tap water. The absorbance was measured at 532nm against a corresponding blank (reaction with all the reagents and treatments except the oil). The standard curve was determined by the TBARs reaction of a series of aliquots

(0.1-1) of 0.2mM 1,1,3,3- tetra-ethoxypropane (Merck S 4258497) prepared in 1-butanol. The results were expressed as μmol malonaldehyde (MDA) kg of oil ($n=3$) (AOCS, 1998.).

STATISTICAL ANALYSIS

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SPSS software program version 16 (one way ANOVA, Tukey). The level of significance was considered ($p<0.05$).

RESULTS

The efficiency of essential oil obtained from fresh leaves was 3% (v/w). Analysis of the isolate by GC and GC-Mass resulted in the identification of Fifty-eight compounds that represented 90.7% of total oil (table 1). In total sum, the major components were α -Pinene (30.2%), 1,8-Cineole (12.1%), Beta-Citronellol (9.6%), Mertenol (6.6%), α -Terpineol (6.1%), 4-Terpineol (5.9%) and Chrysanthenone (2.9%). In the present study, the essential oil showed lower scavenging ability on DPPH radical ($\text{IC}_{50}=147\pm1.50 \mu\text{g/ml}$) than the BHT ($\text{IC}_{50}=4.9\pm0.25\mu\text{g/ml}$). Linoleic acid oxidation was compared with the essential oil and BHT. The concentration of 2g/lit of essential oil and BHT resulted in inhibition values of $50.11\pm1.70\%$ and $95\pm0.25\%$ respectively (table2).

Table 2: Antioxidant activities of the essential oil from the leaves of *P. gnaphalodes* L.

Sample	DPPH IC_{50} value ($\mu\text{g/ml}$)	β -carotene/linoleic acid (% inhibition rate)
Essential Oil	147 ± 1.50	50.11 ± 1.70
BHT	4.9 ± 0.25	95 ± 0.25

Results for the measurement Peroxide value (PV) for control and stabilized samples are revealed in fig. 1. Essential oil at concentration of 400 ppm had the better than effect BHT 100 ppm on oil oxidation. Peroxide value (PV) of soybean oil 800 ppm was comparable to that of BHT 200ppm at all storage periods initially; but decreased after 28th day, suggesting greater stability of BHT200 ppm than essential oil. Peroxide value of essential oil was lower than BHT initially; but became almost equal at the 14th day of analyses, followed by a gradual increase up to the 28th day.

A significant difference ($P<0.01$) in Peroxide value (PV) was observed between the control and soybean oil samples containing synthetic antioxidant, all of which slowed the rate of peroxide formation.

The corresponding inhibition rates after 28th day under accelerated storage conditions compared with the control.

Furthermore, the antioxidant effect of BHT was better than essential oil 800 ppm.

Results for the measurement of TBARs for control and stabilized samples are shown in fig. 2.

Soybean oil 200, 400 and 800ppm concentration revealed behavior similar with a slow degree of increase in formation TBARs up to 21st day of storage and then the rate of formation of TBARs for soybean oil 200 and 400 ppm increased substantially up to 35th day. TBARs values were similar when we used 800 ppm essential oil and 200 ppm BHT in soybean oil.

DISCUSSION

Fresh leaves of *P. gnaphalodes* were collected from east of Iran (Khorasan Rezavi province). The essential oil was prepared by hydrodistillation and was analyzed with GC/Mass.

The yield of *P. gnaphalodes* leaves was 3% (v/w) oil based on dry weight of the sample. The table 1 shows components essential oil was obtained of *P. gnaphalodes* leaves. In total sum, the major ingredient were α -Pinene (30.2%), 1,8-Cineole (12.1%), Beta-Citronellol (9.6%), Mertenol (6.6%), α -Terpineol (6.1%), 4-Terpineol (5.9%) and Chrysanthenone (2.9%). In an investigation reported by Weyerstahl *et al.* (1999) on leaves of *P. gnaphalodes*, 146 components were identified. The most abundant compounds of the essential oil from leaves were α -pinene (34.1), 1,8-Cineole (11.9%), Cadina-1(10%), 4-dien-8a-ol(11%), Terppinen-4-ol (3.1%), Muurola-4,10(14)-dien-8a-ol (3%), α -Terpineol (2.9%), cis-Chrysanthenol (2.3%) and Chrysanthenone (2%). Similar results were observed in composition of *P. gnaphalodes* analyzed in this study (table 1) (Weyerstahl *et al.*, 1999).

Table 2 shows the results of antioxidant activity of the essential oil of *P. gnaphalodes* was evaluated by two different methods, DPPH free radical scavenging and linoleic acid method and DPPH was used to evaluate antioxidant capacity which bleaches purple color when accepting electrons/hydrogen's, thus indicating the inhibition activity (Oke *et al.*, 2009). In the present study, the essential oil showed lower scavenging ability on DPPH ($\text{IC}_{50}=147\pm1.50 \mu\text{g/ml}$) than the BHT ($\text{IC}_{50}=4.9\pm0.25 \mu\text{g/ml}$). The results of Linoleic acid oxidation test indicate that *P. gnaphalodes* essential oil had good antioxidant activity ($50.11\pm1.70\%$); however it was lower than that of BHT (95%).

Oxidation of lipids can also be evaluated more than one method because oxidation occurs through several steps producing various types of products. Peroxide value (PV) is a measure of the concentration of peroxides and hydro peroxides formed in the initial stages of lipid oxidation. Peroxide value (PV) is one of the most widely used tests

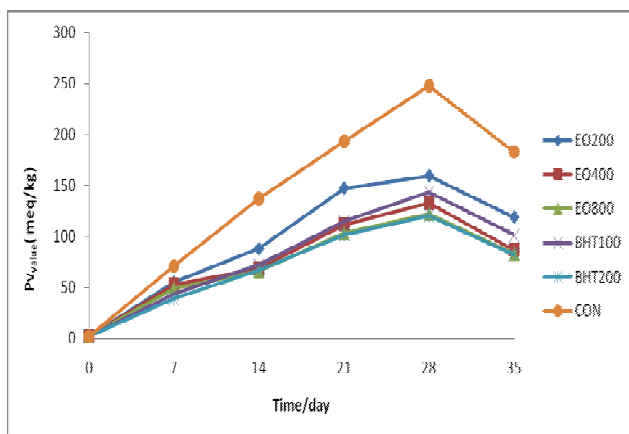


Fig. 1: Effect of *P.gnaphalodes* essential oil and BHT on PV of soybean oil over a 35-day incubation at 65°C. Values were expressed as mean \pm SD of three experiments in three separate experiments.

for measurement of oxidative rancidity in fats and oils. In the present study, oxidation degree of soybean oil samples was determined by measuring Peroxide value (PV) in presence and absence of antioxidants at $65 \pm 3^\circ\text{C}$ for 35 days. Fig. 1 shows the effect of antioxidants during storage on Peroxide value (PV) in samples of the soybean oil.

Results revealed that Peroxide value (PV) after the 14th day increased tremendously in control samples, up to 28th day of analysis, and followed by a sharp decrease on the 28th day. This may be related to the observation of Shahidi *et al.* (1992), which suggest that a decrease in peroxide value after long heating may be due to volatilization of some decay products of lipid hydro peroxides formed in the early stages of oxidation (Shahidi and Wanasundara, 1992). Essential oil at concentration of 400 ppm had better effect than BHT 100 ppm on oil oxidation. Peroxide value (PV) of Soybean Oil 800 ppm was comparable to that of BHT 200ppm at all storage periods initially, but decreased after 28th day. Peroxide value (PV) of essential oil was lower than BHT initially, but became almost equal at the 14th day of analyses, followed by a gradual increase up to the 28th day. A significant difference ($p < 0.01$) in Peroxide value (PV) was observed between the control and soybean oil samples containing synthetic antioxidant.

The inhibition rate after 28 days under accelerated storage conditions compared with the control. Moreover, the antioxidant activities of BHT were better than essential oil in 800 ppm concentration.

Essential oil prevented the formation of TBARS at all concentrations. The thiobarbituric acid (TBA) test is one of the oldest and most frequently used methods for measuring the peroxidation of fatty acid and food. Reaction of malonaldehyde (MDA) with thiobarbituric

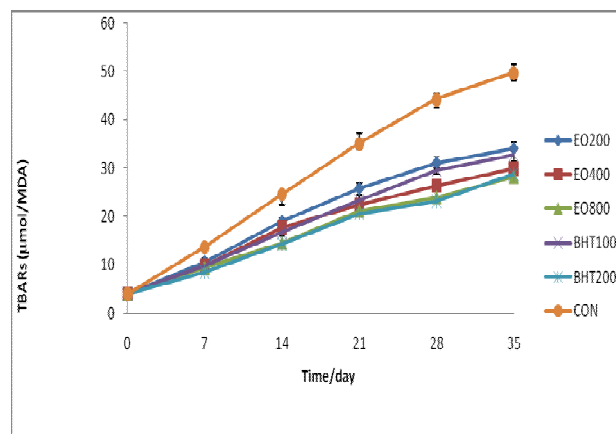


Fig. 2: Effect of *P.gnaphalodes* essential oil and BHT on TBARS values of soybean oil over a 35-day incubation at 65°C. Values were expressed as mean \pm SD of three experiments in three separate experiments.

acid (TBA), pink color was afforded in fats and oils. This color is measured by spectrophotometry at wave length 532nm or by fluorometry at 533nm (Iqbal and Bhanger, 2007).

Antioxidant indices of the samples as well as those of the synthetic antioxidant (BHT) were increased in the absence of the lipid inducer. The effect of antioxidants on TBARS in samples of soybean oil during storage is presented in fig. 2. Steady increase in TBARS diagram was appeared during the storage period. Control group exhibited the highest TBARS at all stages of analyses during storage. To control the amount of TBARS formation was slow at first, while on the 7th day and the duration of storage increased (up to 35 days).

CONCLUSION

The findings of this study showed a powerful antioxidant activity of the essential oil of *P. gnaphalodes*. Therefore it may be used as a food flavor, natural antioxidant and a preventing agent for many diseases caused by free radicals.

REFERENCES

- Ames BM, Shigena MK and Hagen TM (1993). Oxidants, antioxidants and the degenerative disease of aging. *Proc. Natl. Acad. Sci. U.S.A.*, **90**: 7915-7922.
- AOCS (1998). American Oil Chemists' Society. Official method Cd 19-90. 2-Thiobarbituric acid value. Direct method. In: Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th ed. (D. Firestone, ed.) AOCS. Champaign, p.3.
- Burits M and Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res.*, **14**: 323-328.

- Cao Sofic E and prior RI (1996). Antioxidant capacity of tea and common vegetable. *J. Agric. Food Chem.*, **44**: 3426-3441.
- Cuendet M, Hostettmann K and Potterat O (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv Chim. Ac.*, **80**: 1144-1152
- Dapkevicius A, Venskutonis R, Van Beek TA and Linssen PH (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food and Agric.*, **77**: 140-146
- Dauchet L, Amouyet P and Dallongeville J (2005). Fruit and vegetable consumption and risk of stroke. *Neurology*, **65**: 1193-1197.
- Duh PD (1999). Antioxidant activity of water extract of four Harnng Jyur (*Chrysanthemum morifolium* Ramat) varieties in soybean oil emulsion. *Food Chemistry*, **66**: 471-476
- Eshbakova KA (2011). Chemical constituents of *Pulicaria gnaphalodes* Boiss. *Int. J. Phytomed. & Rel. Indus*, **3**(2): 161-163
- Hu FB (2003). Plant-based foods and prevention of cardiovascular disease: An overview. *Am. J. Clin. Nutr.*, **78**(3): 544-551.
- Iqbal S and Bhanger MI (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chem.*, **100**: 246-254
- Kanter M, Meral I, Yener Z, Ozbek H and Demir H (2003). Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by *Nigella sativa* L. in streptozotocin-induced diabetic rats. *Tohoku. J. Exp. Med.*, **201**(4): 213-219.
- Khani A and Asghari J (2012). Insecticide activity of essential oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* against two stored product pests, the flour beetle, *Tribolium castaneum*, and the cowpea weevil, *Callosobruchus maculatus*. *J. Insect Sci.*, **12**:73
- Mozaffarian V (1996). A Dictionary of Iranian Plant Names, Farhang Moaser, Tehran, Iran.
- Official Methods and Recommended Practices of the American Oil Chemists' Society, 3rd edn., edited by R.O. Walker, AOCS, Champaign, IL., p. 1981.
- Oke F, Aslim B, Ozturk S and Altundag S (2009). Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chem.*, **112**: 874-879
- Rădulescu V and Oprea E (2008). Analysis of volatile compounds of officinal *tiliae flos* by gas-chromatography coupled with mass spectrometry. *Farmacia*, **56**(2): 129-138
- Shahidi F and Wanasundara UN (1992). Stability of canola oil and its stabilization by natural antioxidants: A review note. *In Canola Oil*, pp.74-188.
- Shariatifar N (2011). Qualitative and quantitative study of *Pulicaria Gnaphalodes* essential oil and plant extract and evaluation of oxidative stability of soya bean oil in the presence of plant essential oil and extracts. [Thesis] Tehran Uni., [In Persian]
- Velioglu YS, Mazza G, Gao L and Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.*, **46**: 4113-4117.
- Wang H, Cao G and Prior RL (1996). Total antioxidant capacity of fruits. *J. Agric. Food Chem.*, **44**: 701-705.
- Weyerstahl P, Marschall P, Wahlburg HC, Christiansen C, Rustaiyan A and Mirdjalili F (1999). Constituents of the essential oil of *Pulicaria gnaphalodes* (Vent.) Boiss. from Iran. *Flavour Frag. J.*, **14**(2): 121-130.