

## Original Research Article

# Effect of Extract of Aerial Parts of *Urtica dioica* (Urticaceae) on the Stability of Soybean Oil

Mohammad Ali Ebrahimzadeh<sup>1</sup>, Mehdi Gharekhani<sup>2</sup>, Mohammad Ghorbani<sup>2\*</sup> and Pegah Dargany<sup>2</sup>

<sup>1</sup>Traditional and Complementary Medicine Research Center, Mazandaran University of Medical Sciences, Sari, <sup>2</sup>Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

\*For correspondence: **Email:** moghorbani@yahoo.com; **Tel:** +98-151-3543081-3; **Fax:** +98-151-3543084

Received: 13 January 2014

Revised accepted: 10 November 2014

### Abstract

**Purpose:** To evaluate the effectiveness of *Urtica dioica* (Urticaceae) extract as a natural antioxidant and compare with the most commonly used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

**Methods:** Three different *U. dioica* extracts, viz, chloroform, methanol (80 %) and water extracts, were prepared. The antioxidant activity of the extracts were evaluated by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging and soybean oil models. Varying concentrations of the extracts (200, 500 and 800 ppm), BHA and BHT (100 and 200 ppm) were separately added to soybean oil and stored in the oven (60±1 °C) for 25 days. Peroxide and thiobarbituric acid (TBA) values were measured at various heating periods for the oil samples. Total phenolic and flavonoid contents of the extracts were determined using Folin–Ciocalteu and aluminium chloride methods, respectively, while the aerial parts of the plant were also phytochemically screened.

**Results:** Analysis of the chemical composition of *U. dioica* aerial parts showed they contain crude proteins (21.78 %±0.11), crude lipids (1.66 %±0.03), total soluble carbohydrates (37.19 %±0.21), crude fibers (19.62 %±0.14) and ash (19.75 %±0.17). The aqueous extract contained the highest level of total phenolic contents (7.89 ±0.38, mg g<sup>-1</sup> of powder) while the chloroform extract contained the highest level of flavonoid contents (15.40 ±0.53 mg g<sup>-1</sup> powder). The half-maximal concentration (IC<sub>50</sub>) values for chloroform, methanol (80 %) and aqueous extracts in respect of DPPH radical scavenging activity were 77.53±0.99, 199.71±1.02 and 159.88±1.57 µg ml<sup>-1</sup>, respectively. Mixing soybean oil with 200 - 800 ppm of extract decreased oil oxidation and formation rate of TBA reacting substances at a level that is almost equivalent to the synthetic antioxidant, BHT, at a concentration of 200 ppm.

**Conclusion:** These findings demonstrate that mixing soybean oil with *U. dioica* extract can improve the quality of the oil during frying process.

**Keywords:** Antioxidant activity; Soybean oil; DPPH; Peroxide value; Thiobarbituric acid value; *Urtica dioica*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

*Urtica dioica* L. (*U. dioica*) or nettle is a plant belonging to the family of Urticaceae. It is a perennial herb. The stem is erect and green, the

leaves are opposite, finely toothed, dark green above and paler beneath. The small, green, dioecious flowers occur as racemes in the axils of the upper leaves [1]. It is widely used in folk medicine in many countries. *U. dioica* is

traditionally used as antidiabetic and in other disease conditions such as prostatic hyperplasia, inflammation [2], rheumatoid arthritis and allergic rhinitis [3], and as a pivotal therapy in patients with sinusitis [4]. In many ethnobotanical reports, the aerial parts of the plant are recognized as a natural remedy for hypertension [1] and it also possesses many therapeutic effects such as antiviral [5], analgesic [6], and hypolipidemic [7] activities. Anitinoceptive property and locomotor impairment induction by *U. dioica* has been reported recently [9]. *In vitro* anti-oxidant effects of the *U. dioica* have been reported [6]. As food, the young leaf tips are cooked as a spinach-like vegetable, puréed for soup and used to make nettle beer [8]. Raw leaves are highly irritant and recommendations for using them chopped in salads and soft cheeses should be disregarded. Older leaves contain crystals of calcium oxalate, which give a gritty texture, even after cooking. The leaves are dried for tea, which is bland and non-aromatic; it may be added to Indian tea as a tonic. Medicinally, it is used for treating many health problems including anemia, hemorrhage, excessive menstruation, hemorrhoids, arthritis, rheumatism and gout [8]. Its seeds are widely used in folk medicine in many parts of Turkey, in the therapy of advanced cancer patients [10].

Lipid peroxidation is an important reaction that causes deterioration in food during storage and processing. It not only causes loss in food quality but also is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing, and arteriosclerosis [6]. The role of active oxygen and free radicals in tissue damage in such disease states, are increasingly recognized [11]. The most commonly used antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are suspected of causing liver damage and carcinogenesis in laboratory animals. Therefore, the development and utilization of effective antioxidants of natural origin are desired [6].

The aim of the present study was to investigate the antioxidant activity of chloroform, methanol (80 %) and aqueous extracts of *U. dioica* aerial parts using DPPH free radical scavenging and soybean oil models. Phytochemical screening of the plant's aerial parts was also carried out.

## EXPERIMENTAL

### Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemicals

Co (USA). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Vitamin C, Gallic acid and Folin–Ciocalteu reagent and methanol were purchased from Merck (Germany). All other chemicals used were of analytical grade or higher.

### Plant material and preparation of freeze-dried extracts

*U. dioica* aerial parts were collected from Gorgan in May, 2010 and authenticated by Dr Bahman Eslami (Department of Biology, Islamic Azad University of Qaemshahr, Iran). A voucher specimen (No. 91) was deposited in the university's herbarium. Materials were dried at room temperature and coarsely ground before extraction. The dried powdered sample (50 g) was macerated for 24 h successively with 400 ml each of chloroform, methanol (80 %) and water. Extraction was repeated three times and the resulting extracts (for each solvent) were bulked separately and concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried for complete removal of solvent. The yields were 4.4, 20.1 and 22.9 % for chloroform, methanol (80%) and aqueous extractions, respectively.

### Determination of chemical composition

#### Moisture content

*U. dioica* aerial parts (5 g) were dried in an oven at 105 °C to a constant weight and the loss in weight determined as moisture content [12].

#### Ash content

The ash content of *U. dioica* aerial parts was determined by muffle furnace at 550 °C until a constant weight was obtained [12].

#### Crude protein

The total nitrogen of *U. dioica* aerial parts was determined by Kjeldahl method [12]. The crude proteins were then calculated by multiplying the total nitrogen by a factor of 6.25.

#### Total crude lipids

The crude lipids of *U. dioica* aerial parts was determined according to the method of Blight and Dyer [13] using a mixture of chloroform-methanol (2: 1, v/v).

### Crude fiber

*U. dioica* aerial parts (0.5 g) were digested with sulfuric acid (200 mL, 1.25 %), then with sodium hydroxide (200 mL, 1.25 %) and finally washed several times with diethyl ether. The resultant product was dried at 100 °C then ashed at 550 °C [12].

### Soluble carbohydrates

Soluble carbohydrates were determined on dry weight basis by difference.

### Total phenolic content

Total phenolic contents were determined using the Folin–Ciocalteu method [14]. The extract sample (0.5 ml) was mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent for 5 min and 2.0 ml of 75 g l<sup>-1</sup> sodium carbonate was then added. The absorbance of the resulting blue complex was measured at 765 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Methanol was used as control. The content of phenolic compounds was expressed as gallic acid equivalents (mg g<sup>-1</sup> of powder) from a standard curve.

### Total flavonoid content

AlCl<sub>3</sub> method was used for the determination of the total flavonoid content of the sample extracts [14]. The extract in dissolved in MeOH (10 mg/ml, 0.5 ml) was mixed with 1.5 ml of MeOH, 0.1 ml of 10 % AlCl<sub>3</sub>.6H<sub>2</sub>O, 0.1 ml of potassium acetate (1 M) and 2.8 ml of distilled water. The mixture was vigorously shaken and allowed to stand at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. Flavonoid content was expressed as mg quercetin equivalent (mg g<sup>-1</sup> of dry mass) from a standard concentration curve.

### DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extract [15]. Different concentrations of extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature in the dark, the absorbance was recorded at 517 nm. The experiment was replicated thrice. Vitamin C, BHA and quercetin were used as standards. The IC<sub>50</sub> values were calculated from linear regression analysis. IC<sub>50</sub> value denotes the concentration of sample required to scavenge 50 % of DPPH free radicals.

### Peroxide value

Refined, bleached and deodorized (RBD) soybean oil, free from any antioxidant addition was furnished by a GONCHEH vegetable oil refinery (Sari, Iran). Varying concentrations of chloroform (100 %), methanol (80 %) and water (100 %) extracts (200, 500 and 800 ppm), and BHA and BHT (100 and 200 ppm) were tested. In each case, the sample was added to 20 g of soybean oil and stored in an oven (60±1 °C) for 4, 8, 12, 18 and 24 days. Control (blank, i.e., no antioxidant added) sample was also evaluated. The experiment was conducted in duplicate for all treatments. Peroxide value was determined according to AOAC method [12]. A known weight of the oil sample (5 g) was dissolved in a mixture consisting of glacial acetic acid: chloroform (30 mL, 3:2, v/v), and then freshly prepared saturated potassium iodide solution (1 mL) was added. Distilled water (30 mL) was added and titrated slowly with sodium thiosulphate solution (0.1 N) in the presence of starch solution (1 %) as an indicator. Peroxide value was expressed as milliequivalent peroxides kg<sup>-1</sup> oil.

### Thiobarbituric acid value (TBA)

The method of Sidwell *et al* [16] was conducted to determine the TBA value as follows. A known weight of oil (3 g) was dissolved in a carbon tetrachloride (10 mL) followed by the addition of TBA reagent (10 mL, 0.67 % TBA in 50 % acetic acid). The mixture was transferred to a separatory funnel and the aqueous layer was drawn into a test tube and immersed in a boiling water bath for 30 min. The absorbance of the developed pink color was then recorded at 530 nm spectrophotometrically against blank.

### Statistical analysis

Experimental results are expressed as mean±SD. All measurements were carried out in triplicate. Data were subjected to analysis of variance (ANOVA) and least significant difference test in order to compare the mean values of the investigated parameters. IC<sub>50</sub> values were calculated from linear regression analysis.

## RESULTS

### Total phenol and flavonoid contents

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ( $y = 0.0054x + 0.0628$ ,  $r^2 = 0.999$ ). Results are shown in Table 1. Total phenolic contents were in the

range of 2.49 - 7.89 mg gallic acid equivalent (GAE) g<sup>-1</sup> of powder. Aqueous extract with 7.89±0.38 GAE g<sup>-1</sup> of powder showed the highest value. The total flavonoid content was calculated as mg quercetin equivalent per g of powder, by reference to standard curve ( $y = 0.0063x$ ,  $r^2 = 0.997$ ). Total flavonoid contents were in the range of 4.84 -15.40 mg quercetin equivalent (QE) g<sup>-1</sup> of powder. Chloroform extract with 15.40±0.53 QE g<sup>-1</sup> of powder showed the highest value.

### DPPH radical-scavenging activity

In our experiment, the radical-scavenging activity of all extracts increased with increasing their concentrations, i.e. the effect was dose-dependent. IC<sub>50</sub> values were in the range of 77.53-159.88 µg ml<sup>-1</sup> (Table 1). Chloroform extract with highest amount of flavonoid contents showed higher DPPH activity (IC<sub>50</sub> was 77.53±0.99 µg ml<sup>-1</sup>). The IC<sub>50</sub> of BHA, vitamin C and quercetin were 53.93±0.35, 5.05±0.11 and 5.28±0.15 µg ml<sup>-1</sup>, respectively.

### Phytochemical profile of *U. dioica* aerial parts

The phytochemical profile of *U. dioica* aerial parts (on dry weight basis) is shown in Table 2. It shows that *U. dioica* aerial parts contain crude proteins (21.78 %), crude lipids (1.66 %), total soluble carbohydrates (37.19 %), crude fibers (19.62 %) and ash (19.75 %). These data demonstrate that the major constituent of *U. dioica* aerial parts is soluble carbohydrates.

### Peroxide value (PV)

The effect of extracts on changes in peroxide values of soybean oil samples are shown in Table 3. The peroxide values of soybean oil increased significantly ( $p < 0.001$ ) during experimental period, without any additives (control group). The peroxide value of oil without

**Table 2:** Phytochemical profile of dried *U. dioica* aerial parts

Component	Content (%w/w)
Moisture	12.87±0.08
Crude protein	21.78±0.11
Crude lipids	1.66±0.03
Crude fiber	19.62 ±0.14
Ash	19.75±0.17
Soluble carbohydrate	37.19±0.21

Values (mean±SD) are expressed as the mean of three determinations

antioxidant (control group) at the end of 24 days was about 1.35 and 1.57, times as high as that for soybean oil mixed with 100 and 200 ppm of BHA and 1.65 and 2.17 times as high as that for soybean oil mixed with 100 and 200 ppm of BHT, respectively. Addition of extracts (in all concentrations) significantly decreased peroxide value of oil (Table 3). This effect was concentration dependent, i.e. higher concentration shows higher inhibition. On day 24, lowest value (highest potency) was observed in chloroform extract of oil with 800 ppm which was 60.55±3.41. This extract showed the same activity as BHT at the highest concentration used, i.e. 200 ppm (61.53±3.16,  $p > 0.05$ ).

### Thiobarbituric acid value (TBA)

The results of TBA test (Table 4) indicate that thiobarbituric acid value of soybean oil, without any extract or antioxidant (control group) increased significantly ( $p < 0.001$ ) during the experimental period (25 days). Addition of various concentrations of *U. dioica* solvent extracts caused significant ( $p < 0.001$ ) lowering effect in the amount of TBA reacting substances. The effect was dose-dependent. Higher concentrations of extracts or BHA and BHT decreased thiobarbituric acid values. Soybean oil mixed with 800 ppm of chloroform extract had the lowest TBA value at the end of at 25th day.

**Table 1:** Total phenol and flavonoid contents and antioxidant activity of *U. dioica* aerial parts

Extract	Total phenolic contents (mg g <sup>-1</sup> of powder)	Total flavonoid contents (mg g <sup>-1</sup> of powder)	DPPH free radical scavenging, IC <sub>50</sub> (µg ml <sup>-1</sup> ) <sup>a</sup>
Chloroform	2.49 ±0.19 <sup>c</sup>	15.40 ±0.53 <sup>a</sup>	77.53±0.99 <sup>c</sup>
Methanol (80%)	5.30 ±0.27 <sup>b</sup>	5.77 ±0.24 <sup>b</sup>	199.71±1.02 <sup>a</sup>
Water	7.89 ±0.38 <sup>a</sup>	4.84 ±0.21 <sup>b</sup>	159.88±1.57 <sup>b</sup>

<sup>a</sup>IC<sub>50</sub> of BHA, vitamin C and quercetin were 53.93±0.35, 5.05±0.11 and 5.28±0.15 µg ml<sup>-1</sup>, respectively. Values followed by different letter are significantly different at ( $p < 0.05$ )

**Table 3:** Peroxide values (meq. peroxides /kg oil) of *U. dioica* aerial parts

Period (day)	Extraction solvent	<i>U. dioica</i>	<i>U. dioica</i>	<i>U. dioica</i>	BHA	BHA	BHT	BHT	Control
		200‡	500‡	800‡	100‡	200‡	100‡	200‡	
4	Water	4.33±0.17 <sup>b</sup>	4.19±0.05 <sup>b</sup>	4.04±0.93 <sup>b</sup>	4.56±0.82 <sup>b</sup>	4.21±0.93 <sup>b</sup>	4.30±0.47 <sup>b</sup>	4.05±0.69 <sup>b</sup>	6.51±0.35 <sup>a</sup>
	Methanol	4.45±0.34 <sup>b</sup>	4.37±0.04 <sup>b</sup>	4.22±0.28 <sup>b</sup>					
	Chloroform	4.37±0.26 <sup>b</sup>	4.17±0.07 <sup>b</sup>	3.97±0.61 <sup>b</sup>					
8	Water	14.03±0.44 <sup>bc</sup>	12.66±0.87 <sup>cd</sup>	11.08±0.43 <sup>e</sup>	14.37±1.14 <sup>b</sup>	12.24±0.96 <sup>de</sup>	12.18±1.65 <sup>de</sup>	10.90±1.19 <sup>e</sup>	16.94±1.21 <sup>a</sup>
	Methanol	14.34±0.96 <sup>b</sup>	13.42±0.79 <sup>bcd</sup>	12.32±0.36 <sup>de</sup>					
	Chloroform	13.41±0.86 <sup>bcd</sup>	11.80±0.96 <sup>de</sup>	10.93±0.93 <sup>e</sup>					
12	Water	24.12±1.14 <sup>de</sup>	22.17±1.65 <sup>fg</sup>	18.91±1.52 <sup>h</sup>	26.50±1.89 <sup>c</sup>	21.94±1.65 <sup>fg</sup>	21.71±0.97 <sup>g</sup>	17.38±0.88 <sup>i</sup>	35.54±1.24 <sup>a</sup>
	Methanol	28.22±1.67 <sup>b</sup>	25.20±1.25 <sup>cd</sup>	22.11±1.47 <sup>fg</sup>					
	Chloroform	23.23±1.39 <sup>ef</sup>	21.51±1.56 <sup>ag</sup>	15.79±1.06 <sup>g</sup>					
18	Water	56.74±2.67 <sup>c</sup>	51.07±2.67 <sup>f</sup>	48.04±2.32 <sup>hi</sup>	56.74±2.22 <sup>c</sup>	51.03±2.35 <sup>f</sup>	50.99±2.61 <sup>f</sup>	47.09±1.24 <sup>i</sup>	73.07±3.31 <sup>a</sup>
	Methanol	59.41±2.51 <sup>b</sup>	53.37±2.91 <sup>e</sup>	49.56±1.27 <sup>g</sup>					
	Chloroform	54.91±3.30 <sup>d</sup>	48.85±2.14 <sup>gh</sup>	45.07±1.56 <sup>j</sup>					
24	Water	87.99±3.78 <sup>c</sup>	76.22±3.96 <sup>g</sup>	68.65±2.89 <sup>i</sup>	98.46±4.50 <sup>b</sup>	84.76±3.48 <sup>d</sup>	80.58±2.96 <sup>f</sup>	61.53±3.16 <sup>j</sup>	133.5±4.59 <sup>a</sup>
	Methanol	89.27±3.24 <sup>c</sup>	81.60±3.63 <sup>ef</sup>	72.74±2.51 <sup>h</sup>					
	Chloroform	82.85±3.96 <sup>e</sup>	72.32±2.42 <sup>h</sup>	60.55±3.41 <sup>j</sup>					

Values (mean±SD) are expressed as the mean of three determinations; values followed by different letters are significantly different ( $p < 0.05$ ). ‡ Data are in ppm. Refined, bleached and deodorized soybean oil, free from any antioxidant addition, was used as control

**Table 4:** Thiobarbituric acid value (mg malonaldehyde /;g oil) of *U. dioica* aerial parts

Period (day)	Extraction solvent	<i>U. dioica</i>	<i>U. dioica</i>	<i>U. dioica</i>	BHA	BHA	BHT	BHT	Control
		200‡	500‡	800‡	100‡	200‡	100‡	200‡	
5	Water	0.052±0.008 <sup>b</sup>	0.046±0.002 <sup>bcd</sup>	0.043±0.001 <sup>bcd</sup>	0.054±0.003 <sup>b</sup>	0.052±0.002 <sup>bc</sup>	0.045±0.003 <sup>bcd</sup>	0.041±0.002 <sup>d</sup>	0.069±0.004 <sup>a</sup>
	Methanol	0.054±0.004 <sup>b</sup>	0.047±0.006 <sup>bcd</sup>	0.045±0.003 <sup>bcd</sup>					
	Chloroform	0.049±0.001 <sup>bcd</sup>	0.044±0.004 <sup>bcd</sup>	0.041±0.006 <sup>cd</sup>					
10	Water	0.085±0.003 <sup>b</sup>	0.075±0.005 <sup>cde</sup>	0.067±0.004 <sup>ef</sup>	0.080±0.001 <sup>bcd</sup>	0.076±0.002 <sup>bcd</sup>	0.074±0.005 <sup>cde</sup>	0.066±0.004 <sup>ef</sup>	0.111±0.008 <sup>a</sup>
	Methanol	0.084±0.007 <sup>bc</sup>	0.077±0.004 <sup>bcd</sup>	0.072±0.003 <sup>def</sup>					
	Chloroform	0.082±0.006 <sup>bcd</sup>	0.072±0.001 <sup>def</sup>	0.063±0.004 <sup>f</sup>					
15	Water	0.119±0.014 <sup>bc</sup>	0.110±0.024 <sup>cde</sup>	0.107±0.009 <sup>de</sup>	0.125±0.008 <sup>b</sup>	0.120±0.006 <sup>bc</sup>	0.117±0.008 <sup>bcd</sup>	0.107±0.014 <sup>de</sup>	0.153±0.009 <sup>a</sup>
	Methanol	0.124±0.005 <sup>b</sup>	0.113±0.003 <sup>cde</sup>	0.111±0.005 <sup>cde</sup>					
	Chloroform	0.117±0.001 <sup>bcd</sup>	0.109±0.003 <sup>de</sup>	0.105±0.007 <sup>e</sup>					
20	Water	0.224±0.008 <sup>bcd</sup>	0.173±0.007 <sup>fgh</sup>	0.156±0.004 <sup>gh</sup>	0.183±0.006 <sup>b</sup>	0.169±0.004 <sup>cde</sup>	0.165±0.003 <sup>def</sup>	0.152±0.005 <sup>gh</sup>	0.224±0.009 <sup>a</sup>
	Methanol	0.179±0.006 <sup>bc</sup>	0.159±0.005 <sup>efg</sup>	0.156±0.006 <sup>fgh</sup>					
	Chloroform	0.172±0.007 <sup>cd</sup>	0.152±0.006 <sup>gh</sup>	0.146±0.004 <sup>h</sup>					
25	Water	0.222±0.017 <sup>bc</sup>	0.201±0.014 <sup>ef</sup>	0.185±0.002 <sup>gh</sup>	0.227±0.014 <sup>bc</sup>	0.208±0.014 <sup>de</sup>	0.197±0.017 <sup>f</sup>	0.176±0.009 <sup>hi</sup>	0.329±0.027 <sup>a</sup>
	Methanol	0.231±0.018 <sup>b</sup>	0.210±0.007 <sup>de</sup>	0.195±0.008 <sup>fg</sup>					
	Chloroform	0.219±0.006 <sup>cd</sup>	0.198±0.009 <sup>f</sup>	0.168±0.004 <sup>i</sup>					

Values (mean±SD) are expressed as the mean of three determinations; values followed by different letters are significantly different ( $p < 0.05$ ); ‡ data are in ppm. Refined, bleached and deodorized soybean oil, free from any antioxidant addition used as control

## DISCUSSION

Total phenol compounds determined by Folin Ciocalteu method by reference to standard curve. Aqueous extract contained higher level of total phenolic contents than other extracts. The total flavonoid content was calculated as mg quercetin equivalent/g of powder by reference to standard curve. Chloroform extract contained higher level of flavonoid contents (15.40±0.53 mg gallic acid equivalent g<sup>-1</sup> of powder) than other extracts. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant

antioxidant activities. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [14].

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [17]. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [14]. The model of scavenging the stable DPPH radical is a widely used method to

evaluate antioxidant activities in a relatively short time compare to other methods. The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants [14]. This is visualized as a discoloration from purple to yellow.

Chloroform extract with higher amount of flavonoid contents showed higher activity. Oil oxidation can be determined by different methods such as peroxide value, volatile compounds, oxygen depletion, conjugated diene content and flavour score [18]. The general principle of most of the methods is the liberation of iodine from potassium iodide in an acid medium. Peroxides are the primary products of lipid oxidation; therefore, determination of these compounds can be used as oxidation index for the early stages of lipid oxidation [19].

The peroxide values of soybean oil increased significantly ( $p < 0.001$ ) during experimental period, without any additives. No significant differences were observed between this extract and that sample treated with 200 ppm of synthetic antioxidant BHT, as the most potent antioxidant in our study. The rank of potency in inhibition of peroxidation was in the order of chloroform extract > aqueous extract > methanol extract.

Chloroform extract contained highest amount of flavonoids. It seems in this plant, flavonoids have major role in oil peroxidation inhibition. There are two stages of oil oxidation, i.e., the first stage is the formation of hydroperoxides and the second one is the decomposition of hydroperoxides to produce secondary oxidation products which could be react with TBA reagent to produce colored compounds which absorb usually at 530 nm [20].

The results of TBA test indicate that addition of various levels of *U. dioica* aerial parts extract (for all extraction solvents) caused significant lowering effect in the amount of TBA reacting substances. Here again, the results of TBA value of soybean oil indicted the powerful antioxidant activity of polyphenolic compounds present in *U. dioica* extract. The rank order of extract in terms of the capacity to decrease oil oxidation and the formation rate of TBA reacting substances was chloroform extract > aqueous extract > methanol extract. Chloroform extract contained the highest amount of flavonoids. It seems in this plant, flavonoids have major role in oil peroxidation inhibition. The results also show that the natural polyphenolic compounds of found in chloroform *U. dioica* extract (800 ppm) exhibited antioxidant

effect equivalent to that of synthetic standard antioxidant (BHT, 200 ppm).

Antioxidant activity of methanolic extract of peanut hulls has been evaluated in soybean oil after accelerated oxidation at 60 °C. Results showed that the oil with 0.12, 0.48, and 1.20 % extract had significantly ( $p < 0.05$ ) lower peroxide values than the control after storage at 60 °C. Oils with 0.48 and 1.20 % extract were significantly ( $p < 0.05$ ) superior to 0.02 % BHA in reducing oxidation of oil [21]. Antioxidant activities of the water extract of the four Harnj Jyur (*Chrysanthemum morifolium* Ramat) varieties have been evaluated in soybean emulsion after an accelerated oxidation at 60 °C, which were examined by peroxide values and 2-thiobarbituric acid tests. Results showed that all extracts were significantly ( $p < 0.05$ ) more stable than the control. 0.02 % of each extracts had much more and better antioxidative properties than 0.02 % of BHA [22]. Methanol extract of Noble oats has been tested in soybean at 60 °C. During storage, the peroxide values of the oils were generally significantly lower ( $p \leq 0.05$ ) with the addition of the extract than was the control (no additives), and peroxide values were slightly higher than for oils containing TBHQ.

## CONCLUSION

These findings demonstrate that mixing soybean oil with 200 - 800 ppm of organic solvent extract of *U. dioica* aerial parts decreased oil oxidation and the formation rate of TBA reacting substances at a level equivalent to that of synthetic antioxidant. Consequently, mixing oil with natural compounds of *U. dioica* may improve the quality of the oil during frying due to the role of flavonoids in oil peroxidation inhibition.

## ACKNOWLEDGEMENT

This research was supported partially by a grant from the Gorgan University of Agricultural Sciences and Natural Resources. Special thanks to Dr. Bahman Eslami for authenticating the plant scientific name. This work was MS.C thesis of M. Gharekhani.

## REFERENCES

1. Testai L, Chericoni S, Calderone V, Nencioni G, Nieri P, Morelli I, Martinotti E. Cardiovascular effects of *Urtica dioica* L. (*Urticaceae*) roots extracts: *in vitro* and *in vivo* pharmacological studies. *J Ethnopharmacol* 2002; 81: 105-109.
2. Krzeski T, Kazon M, Bordowski A, witeska A, Kuczera J. Combined extracts of *Urtica dioica* and *pygeum* *Trop J Pharm Res, January 2015; 14(1): 130*

- africanum* in treatment of benign prostatic hyperplasia: double-blind comparison of two doses. *Clin Therapeut* 1993; 15: 1011-1020.
3. Miltman P. Randomized double blind study of freeze dried *Urtica dioica* in the treatment of allergic rhinitis. *Planta Medica* 1990; 56: 44-47.
  4. Helms S, Miller A. Natural treatment of chronic rhinosinusitis. *Altern Med Rev* 2006; 11(3): 196-207.
  5. Uncini Manganelli REL, Tomei PE. Antiviral activity in vitro of *Urtica dioica* L., *Parietaria diffusa* M. and *Sambucus nigra* L. *J Ethnopharmacol* 2005; 98(3): 323-327.
  6. Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J Ethnopharmacol* 2004; 90: 205-215.
  7. Daher CF, Baroody KG, Baroody GM. Effect of *Urtica dioica* extract intake upon blood lipid profile in the rats. *Fitoterapia* 2006; 77(3): 183-188.
  8. Turgay EÖ. Antibacterial activities of some plant extracts used in folk medicine *Pharm Biol* 2002; 40 (4): 269-273.
  9. Mahmoudi M, Ebrahimzadeh MA, Pourmorad F, Yasini S. Antinociceptive and locomotor impairment by methanolic extract of *Urtica Dioica*. *Int J Biol Biotech* 2007; 4(2-3): 181-185.
  10. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of *Nigella sativa* L and *Urtica dioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World J Gastroenterol* 2005; 11(42): 6684-6688.
  11. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med* 1991; 91: 14-22.
  12. AOAC. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemistry, Arlington, Virginia, USA, 2000.
  13. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; 37: 911-917.
  14. Nabavi SF, Ebrahimzadeh MA, Nabavi SM, Eslami B. Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. *Grasas y Aceites* 2010; 61(3): 244-250.
  15. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B. Antioxidant activity of *O. Sintenisii* bulbs and aerial part at flowering stage. *Trop J Pharm Res* 2010; 9(2): 141-148.
  16. Sidwell CG, Salwin H, Benca M, Mitchell JH. The use of thiobarbituric acid as a measure of fat oxidation. *J Am Oil Chem Soc* 1954; 31: 603-606.
  17. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of *H. officinalis* L. Var. *angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*. *Pak J Pharm Sci* 2010; 23(1): 29-34.
  18. Dunlap FG, White PJ, Pollak LM, Brumm TJ. Fatty acid composition of oil from adapted, elite corn breeding materials. *J Am Oil Chem Soc* 1995; 72: 981-987.
  19. Barthel G, Grosch W. Peroxide value determination comparison of some methods. *J Am Oil Chem Soc* 1974; 51: 540-544.
  20. Orthoefer FT, Cooper DS. Evaluation of used frying oil. In: Perkins, EG, Erickson, MD., editors. *Deep-Frying Chemistry, Nutrition and Practical Application*, AOAC Press, Champaign; 1996; pp 285-296.
  21. Duh P, Yen G. Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. *J Am Oil Chem Soc* 1997; 74: 745-748.
  22. Duh P. Antioxidant activity of water extract of four Harng Jyur (*Chrysanthemum morifolium* Ramat) varieties in soybean oil emulsion. *Food Chem* 1999; 66: 471-476.
  23. Tian LL, White PJ. Antioxidant activity of oat extract in soybean and cottonseed oils. *J Am Oil Chem Soc* 1994; 71(10): 1079-1086.