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Full Length Research Paper

Antioxidative effects of Iranian *Urtica dioica* L. extracts on the oxidation of sunflower oil

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The aim of the present study was to evaluate antioxidative effects of water and ether extracts of Iranian *Urtica dioica*, during its pre and post flowering on vegetable oil during storage. Different concentration of water and ether extracts (0, 200, 400, 600, 800 and 1000 ppm) and β-Hydroxy toluene (BHT; 200 ppm) were added to sunflower oil emulsion in the presence of cupric ions and incubated for 7 days at 60°C. Peroxide values (PVs) and thiobarbituric acid reacting substances (TBARS) levels were measured each day up to day seven. Furthermore, antioxidant capacity of the extracts was determined using DPPH and β-Carotene linoleic acid methods. Values were compared among groups in each incubation time points using 1-way ANOVA. Results showed that DPPH and β-Carotene-linoleic acid assay findings on the *U. dioica* extracts were comparable to those found for BHT. Furthermore, in all incubation time points, *U. dioica* extracts lowered PVs and TBARS levels significantly when compared with the control ($p<0.001$). In this respect, the water extract was more potent than the ether extract. In addition, antioxidant properties of the water extracts in post flowering stage were similar to those of BHT in the same concentration (200 ppm). It seems that water extract of the post flowering *U. dioica* is a potent antioxidant for oil and oily products.

Key words: *Urtica dioica*, antioxidant activity, sunflower oil emulsion, extract.

INTRODUCTION

Lipid oxidation can lead to development of unpleasant rancid or off flavors as well as nutritional value loss. In addition, oxidation conducts formation of potentially toxic end- products which can cause health disorders such as atherosclerosis and cancer amongst the rest (Koleva et al., 2003). Lipids oxidation is one of the main causes of deterioration throughout raw material storage, processing, heat treatment and further storage of final products (Gachkar et al., 2007; Koleva et al., 2003; Pizzale et al., 2002). Effective antioxidants are added to foods in order to retard or reduce this kind of decline. Synthetic antioxidants have been used for a long time, but their function has recently come into dispute due to a suspected carcinogenic potential and the general rejection of synthetic food additives by consumers. Thus, there is a developing appeal to verificate and introduce natural antioxidants as alternatives to the synthetic compounds (Botsoglou et al., 2002; Castenmiller et al., 2002; Kaur and kapoor, 2001; Koleva et al., 2003; Pizzale et al., 2002). Epidemiological researches have proved association of frequent consumption of vegetables and fruits with a lower risk of cardiovascular disease and cancer, so, natural antioxidants which exist mainly in these kinds of nourishments have gained increasing interest among consumers (Castenmiller et al., 2002; Javanmardi et al., 2003; Kaur and Kapoor, 2001).

Phenolic and polyphenolic compounds are considered as one of the most important groups of plant secondary metabolites, composed of phenolic or polyphenolic compounds comprising an integral division of the nourishment with considerable quantities in vegetables, fruits and beverages (Bahramikia and Yazdanparast, 2008). Polyphenols possess antioxidant activity chiefly

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due to their redox properties. These combinations perform mainly as free radical terminators, hydrogen donors and metal chelators (Yingming et al., 2007).

Nettle (*Urtica dioica L.*) as an annual and perennial herb, has already been known and therefore consumed for a long time as a medicinal plant in many parts of the world. Nettle's isolated major flavonoid glycosides have been determined to be immune stimulatory, anti-carcinogenic, anti-inflammatory, anti-oxidant and anti-allergenic activities (Yener et al., 2009).

The purpose of the present study was to evaluate the in vitro antioxidative properties of the water and ether extracts of Iranian nettle during two different conditions of pre and post flowering stages on the lipid peroxidation of sunflower oil emulsion.

MATERIALS AND METHODS

Plant material and extraction

Aerial parts of *U. dioica* were collected from Mazandaran province (Iran) during the pre and post flowering phases in spring and summer of 2009. A voucher specimen for this plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University, Tehran, Iran. Dried aerial parts of the plant were prepared in the dark, grounded in a grinder and stored at refrigerator (4°C) until use. Refined, bleached and deodorized sunflower oil without any antioxidants was taken from a manufacturing company (Ghoo Oil Factory, Tehran, Iran).

Two types of extracts were employed in the present study (water and ether). Ether extract was prepared according to the method of Shyamala et al. (2005) with some modifications. Briefly, 15 g of dried and chopped leaves was extracted with 100 ml diethyl ether (Merck Art.1773) for 24 h with occasional shaking. Water extract was prepared by means of a soxhlet. In this regard, leaf samples were extracted with distilled water in a soxhlet apparatus until extraction water became colorless. Both extracts were further filtered and evaporated to dryness in a vacuum dryer (Rotary evaporator, RE-52AA, China).

Antioxidant activity

DPPH assay

Bleaching of purple colored methanol solution of DPPH was applied to determine the capability of the related extracts and some pure compounds regarding hydrogen atom or electron donation. This spectrophotometric assay treats constant radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Burits and Bucar, 2000; Cuendet et al., 1997). In short, different concentrations (50 µl) of the extracts, prepared in water and ether, were added to 5 ml of a 0.004% methanol solution of DPPH (Sigma D9132). The absorbance was read against a blank at 517 nm after 30 min incubation at room temperature. Then the following formula was applied to calculate the inhibition free radical DPPH in percent;

$$(I\ \%): (A_{blank} - A_{sample}/ A_{blank}) \times 100$$

A_{blank} is representative for the absorbance of the control reaction (including all reagents except the test compounds) and A_{sample} points to the absorbance of the test compounds. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentages against extract concentration using PHARM/PCS-version 4. All tests were done in triplicate. Values (mean ± SD) of the extracts were compared with those of BHT using student's t-test. α in all cases was 0.05 ($p<0.05$).

β-Carotene-linoleic acid assay

Antioxidant capacity was determined measuring the volatile organic compounds inhibition and the conjugated diene hydroperoxides arising from linoleic acid oxidation according to the method of Dapkevicius et al. (1998). In this respect, a stock solution of β -carotene-linoleic acid mixture was prepared as follow: 0.5 mg β -carotene (Merck, K15555836) was dissolved in 1 ml of chloroform (HPLC grade) and 25 µl linoleic acid (Sigma, L1376-500MG) and then 200 mg Tween 40(Merck, 822185) was added. After evaporation of chloroform, 100 ml oxygen-saturated distilled water was added with vigorous shaking. Then, 2500 µl aliquots were dispensed into the test tubes and 350 µl of the extract (2 g/l) was added to the tubes and the resulted emulsion systems were incubated for 48 h 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, Antioxidative capacities of the extracts were compared with those of BHT and blank. Further, all inhibition percentages were compared using 1-way ANOVA. α was 0.05.

Antioxidant activity in sunflower oil emulsion

Antioxidative effects of the extracts on lipid peroxidation were evaluated in the sunflower oil-in-water emulsion (50:50, w/v) employing the method of Duh (1999). $CuSo_4$ (Merck, 2787) solution (2.26 mM) was added to the emulsion to give a final copper concentration of 55 µmol/kg just before the oxidation assay. Each emulsion sample (4 ml) was transferred into a series of capped glass test tubes, then, *U. dioica* extracts taken in both stages of pre and post-flowering (0, 200, 400, 600, 800, 1000 ppm) and BHT (200 ppm) were added to the test tubes and then put in a dark oven at 60°C for thermal oxidation. Peroxide values (PVs) and thiobarbituric acid reacting substances (TBARS) levels were measured in each 24 h during 7 days. PVs were measured using AOCS Official Method Cd 8 to 53 (1990). For this purpose, a known weight of edible oil sample (3 g) was dissolved in glacial acetic acid (30 ml) and chloroforms (20 ml) and, saturated KI solution (1 ml) was added afterward. The mixture was kept in a dark place for 1 min. After the addition of distilled water (50 ml), the mixture was titrated against sodium thiosulphate (0.02 N) using starch as an indicator. A blank titration was done parallel to the treatment and PVs (meq of oxygen/ kg) were calculated using the following formula: peroxide value = 1000 S N/W. In this formula, S represents the volume of sodium thiosulphate solution (blank corrected) in ml; N indicates the normality of sodium thiosulphate solution (0.02 N) and W shows the weight of oil sample (g).TBARS values were determined daily by the method of McDonald and Hultin (1987).In this respect, emulsion (0.05 ml) combined with 0.95 ml of water and 2.0 ml of TBA reagent (15 g of trichloroacetic acid, 0.375 g thiobarbituric acid, 2 ml HCL and 82.9 ml of distilled water) in the test tubes and incubated in a boiling water bath for 15 min. The absorbance spectra were obtained at 532 nm. Concentrations of TBARS were determined by the standard curve using 1, 1, 3, 3-tetraethoxypropane (Merck, S4258497) as the standard.

Statistical analysis

PVs and TBARS values of the pre and post-flowering stages were compared using pared t-test. Meanwhile, for each case, IC_{50} was estimated and compared among different time points.

Table 1. *In vitro* antioxidant activities of *U. dioica*, water and ether extracts (in pre and post flowering stages) and BHT in DPPH assay.

Sample	DPPH (µg/ml)
Water extract (post flowering)	8.4 ± 0.1
Water extract (pre flowering)	11.7±0.2
Ether extract (post flowering)	12.4±0.1
Ether extract (pre flowering)	22.5±0.2
BHT	5±0.2

IC50 values were calculated and expressed as mean ±SD.

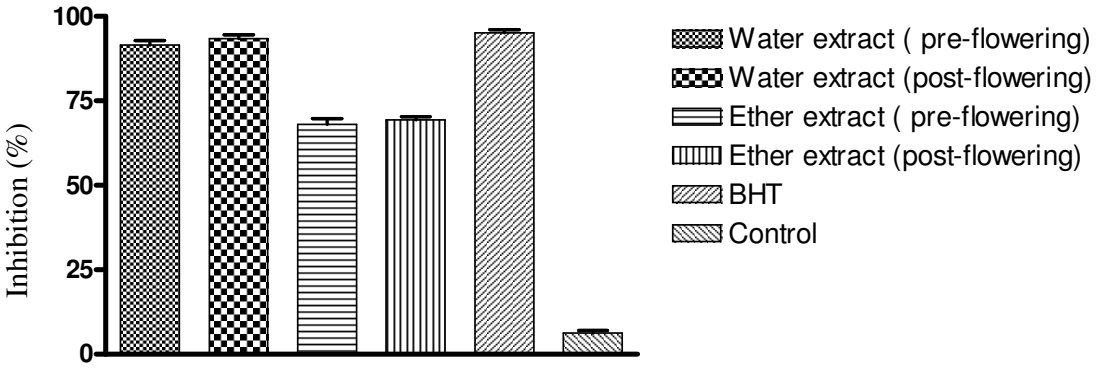

Figure 1. Antioxidant activity of *U. dioica* extracts defined as inhibition percentage through β-carotene - linoleic acid assay.

RESULTS

Free radical - scavenging activity

The amount of extract which induced 50% inhibition (IC_{50}) is presented in Table 1. The obtained results show that both water and ether extracts of the *U. dioica* decrease the values of free radicals as a dose-dependent manner. While both water and ether extracts of the nettle show higher values than that of BHT, the antiradical effects of the water extracts are more potent than those of ether; however, in both cases, post-flowering stage show more potency in comparison with the pre-flowering stage ($p<0.001$). According to the above mentioned, the difference between water extract of nettle in post-flowering stage and BHT is very feeble.

Inhibitory effect of extracts on lipid peroxidation

Figure 1 displays inhibition of lipid peroxidation in response to water and ether extracts in pre and post flowering stages. As it can be seen, 91.6 and 93.4% inhibition were obtained as a result of pre and post-flowering stages water extracts of nettle respectively, while, BHT showed 95% of inhibitory effect. In addition, inhibitory potency of nettle ether extract in pre and post-flowering stages were 68 and 69.3%, respectively. Both pre and post-flowering stages of nettle water extracts showed higher inhibition (91.6 and 93.4%) than those of ether (68 and 69.3%).

Effects of extracts on the oxidative parameters of sunflower oil emulsion

The effects of water and ether extracts of the pre and post-flowering stages on the PVs and TBARS levels within 7 days of incubation at 60°C are shown in Figures 2 to 5. The calculated values of PV and TBARS levels were obtained to be 1.4 meq kg^{-1} and 22.5 nmol kg^{-1}, respectively in zero time. All concentrations of the extracts decreased the PVs and TBARS levels significantly in comparison with the control ($p< 0.001$).

Figure 2a shows alteration in PV values during 7 days of incubation. We have found that 200 ppm of nettle water extract in post-flowering stage effectively decreases PV values (as much as BHT). Furthermore, higher concentrations of the water extract (400, 600, 800 and 1000 ppm) made more decrease in PV values than those of BHT ($p< 0.001$). On the other hand, TBARS values (Figure 2b) of the water extract were significantly

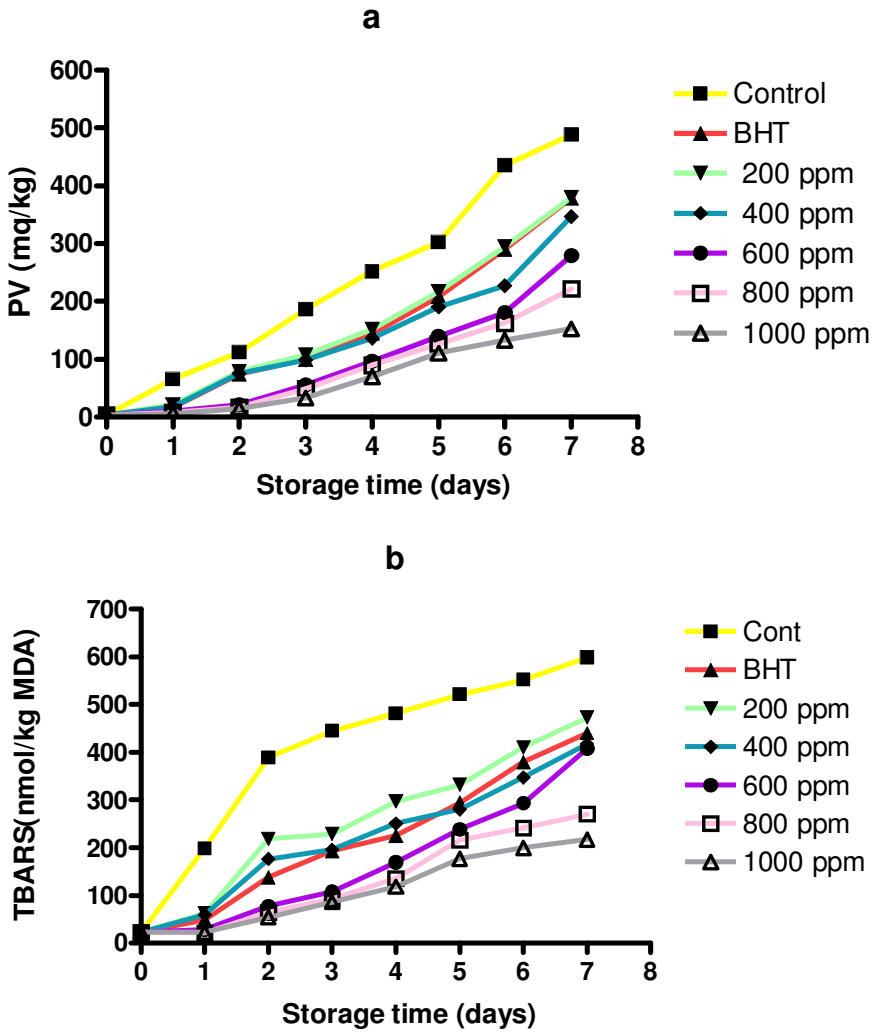

Figure 2. Effect of *U. dioica* post flowering water extract and BHT on PVs (a) and TBARS levels (b) of sunflower oil emulsion over a 7 day incubation at 60°C. Values were expressed as mean ± SD of three experiments in three separate experiments.

decreased compared to the control in 7 days of incubation period. Accordingly, while 200 ppm of the water extract showed a potency as effective as BHT, higher concentrations of the water extract showed more potency than the allowed BHT concentration (200 ppm) as a food additive ($p< 0.05$).

Figure 3 indicates PVs and TBARS values in emulsion of sun flower oil mixed with pre- flowering water extract. It has been shown in Figure 3a that 400 ppm of nettle water extract in pre-flowering stage notably decreased PV as much as BHT ($p<0.05$). In addition, higher concentrations of the pre-flowering water extract (600, 800 and 1000 ppm) made more decrease in PV values than those of BHT ($p<0.001$). High concentrations of pre-flowering water extract (600, 800 and 1000 ppm) also indicated significant lower levels of TBARS than those of BHT group ($p<0.001$) from the fourth day till the end of the study, while there were no considerable difference between the groups of BHT and 200 ppm (Figure 3b).

The parameter rates of PV and TBARS related to emulsion containing post-flowering ether extract are presented in Figure 4. It was found that 600 ppm of nettle ether extract in post-flowering stage effectively decreased PV value, more than BHT ($p<0.005$). In this respect, there was no drastic difference between groups of BHT and 400 ppm ($p>0.05$).On the other hand, TBARS values (Figure 4b) of ether extract in post-flowering stage showed more antioxidative potency in higher concentrations (800 and 1000 ppm) compared to those of BHT ($p<0.05$). Regarding this, there was no significant difference between 600 ppm of post-flowering ether extract and BHT ($p>0.05$).

Figure 5a shows alteration in PV values of pre-flowering stage ether extract during 7 days of incubation.

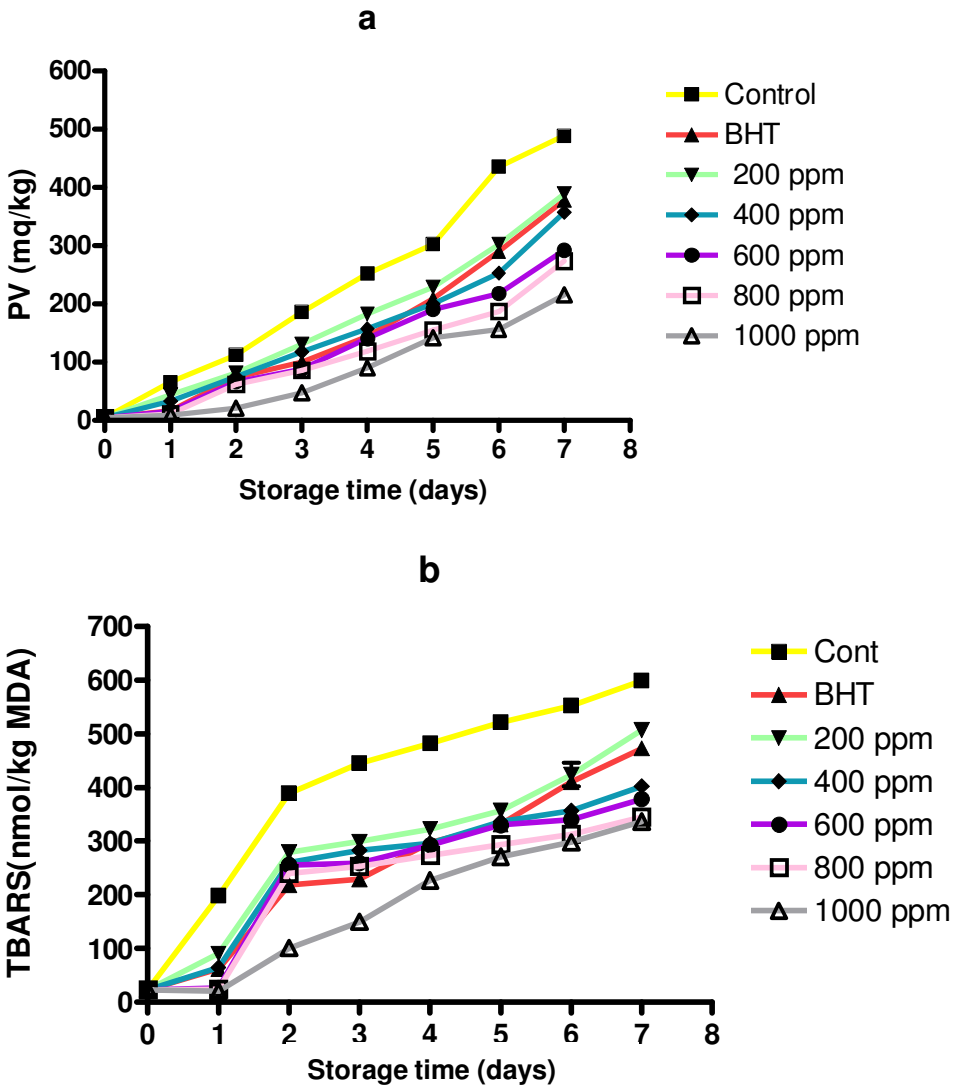

Figure 3. Effect of *U. dioica* pre flowering water extract and BHT on PVs (a) and TBARS levels (b) of sunflower oil emulsion over a 7 day incubation at 60°C.Values were expressed as mean ±SD of three experiments in three separate experiments.

It was seen that 1000 ppm of nettle ether extract effectively decreased PV more than that of BHT($p<0.001$); therefore, it can be concluded that, antioxidative potency of BHT concentration (200 ppm) is more than 200, 400 and 600 ppm concentrations of nettle ether extract in pre-flowering stage ($p<0.05$). Furthermore, TBARS values of oil supplemented with pre-flowering ether extract and BHT are similar (Figure 5b).

DISCUSSION

Decrease in DPPH radical scavenging activity due to both water and ether extracts of *U.dioica* in pre and post flowering stages were higher than those reported by Gulcin et al. (2004) on the water extract (> 60 µg/ml) and Mavi et al. (2004) on the methanol extract (335 µg/ml) of *U. dioica* which difference might be attributed to the regional plant varieties.

On the other hand, we have found more inhibition in oxidation values for both pre and post-flowering stages than those reported by Hudec et al. (2007) for nettle water extract (76%). However, inhibitory effects of nettle ether extracts on oxidation were lower than those reported by Hudec et al. (2007) for both pre and post-flowering stages. In this regard, inhibitory effect of *U. dioica* water extract was similar to that of BHT. Gulcin et al. (2004), Proestos et al. (2006) and Hudec et al. (2007) argued that such properties of *U. dioica* water and ether extracts can be attributed to its phenolics (such as, gallic acid, syringic acid, ferulic acid) and flavonoids (such as, catechin hydrate and epicatechin) constituents.

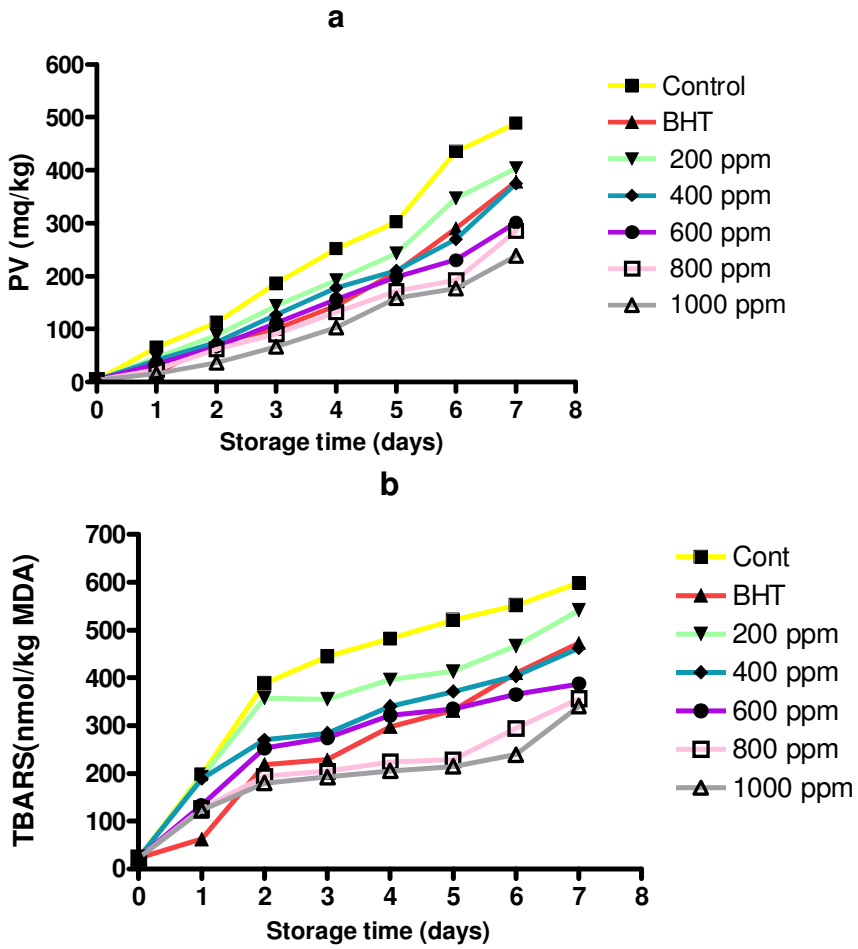

Figure 4. Effect of *U. dioica* post flowering ether extract and BHT on PVs (a) and TBARS levels (b) of sunflower oil emulsion over a 7 day incubation at 60°C. Values were expressed as mean ±SD of three experiments in three separate experiments.

Accordingly, Luximon-Ramma et al. (2002) showed linear correlation between antioxidant activity and phenolic contents of plant, fruits and beverages extracts. Sugihara et al. (1999) and Spencer (2008) discussed that flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals. Inhibition of lipid peroxidation may be mainly related to phenolic compounds of plants such as flavonoids and phenolic acid in polar extract of plants. Rahmat et al. (2003) and Arumugam et al. (2006) argued that these extracts have more beneficial effects on free radicals in comparison with the non-polar ones. On the other hand, some researches have shown that the presence of high α-tocopherol and nitrate levels in summit of the plants during flowering period may result in their potent antioxidant effects (Bahmann et al., 2006).

At the present study, we have shown that water and ether extracts of *U. dioica* are able to inhibit both primary and secondary oxidation of sunflower oil during storage compared to the control. Because of the presence of different antioxidants in different leafy plants, their hydro or organic extracts are able to inhibit both lipid peroxidation and scavenging free radicals. On the other hand, it is believed that synergism among different antioxidant compounds of an extract defines its antioxidant properties. In this regard, Duh et al. (1999) compared antioxidative activities among water extracts of Harng Jyur varieties, tocopherol and butylated hydroxyanisol (BHA) in soy bean oil emulsion through measuring both primary and secondary oxidation. They showed high inhibition in both primary and secondary oxidation for the water extract. They attributed this property to the presence of several polyphenolic antioxidants with a range of solubilities and synergism among them. Sikwese et al. (2007) compared antioxidative effects of sorghum crude phenolic extract with TBHQ (synthetic antioxidant) in sunflower oil in the presence of ferric ions. They showed that TBHQ caused a higher inhibitory effect on primary oxidation of oil than the extract yet their abilities for inhibiting the secondary oxidation were similar. They discussed that such characteristics may be due to disability of TBHQ in

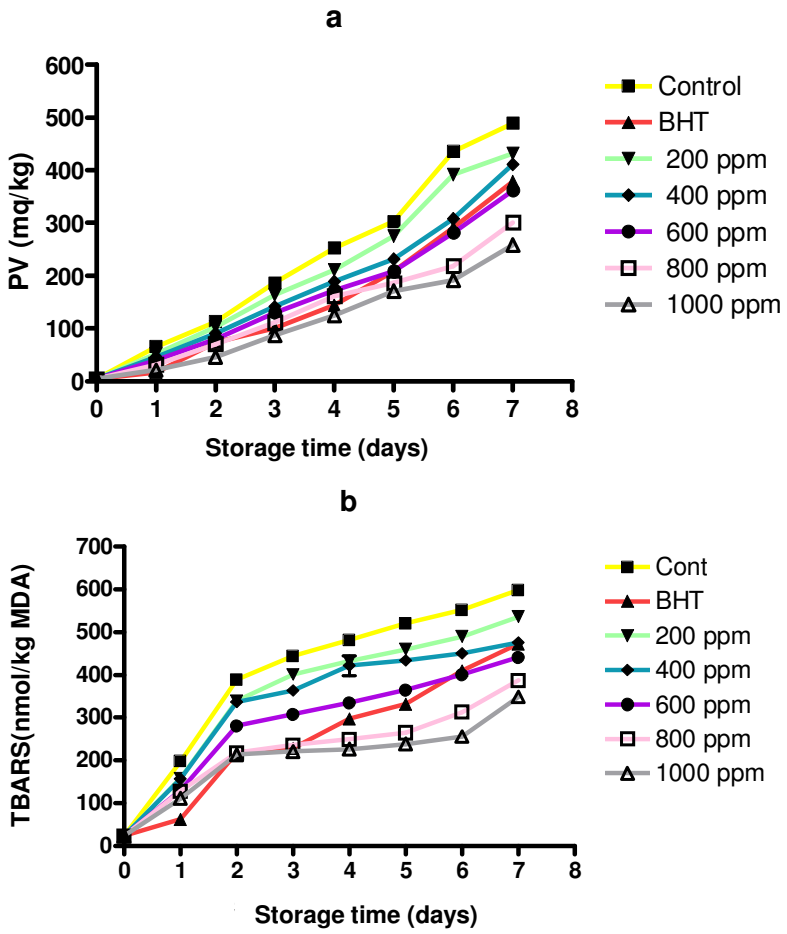

Figure 5. Effect of *U. dioica* pre flowering ether extract and BHT on PVs (a) and TBARS levels (b) of sunflower oil emulsion over a 7 day incubation at 60℃.Values were expressed as mean ± SD of three experiments in three separate experiments.

chelating of metal ions compared to the phenolic compounds. Shyamala (2005) showed that free radical scavenging activities of the ethanol extract for some leafy vegetables (such as cabbage, coriander leaves, hongone and spinach) were lower than BHA. However, they showed that during storage, their antioxidative effect on heated sunflower oil and groundnut oil was higher than BHA. Such pattern was shown by Kamkar et al. (2010) on both water and methanol extracts of *Mentha pulegium* compared to BHT when added to sunflower oil emulsion. This property can be due to the presence of water soluble active components like flavonoid, terpenoid and polyphenol in the leafy part of the vegetables (Arumugam et al., 2006; Rahmat et al., 2003).

In conclusion, we have shown that *U.dioica* extract can be considered as an antioxidant agent to be added to edible oils. In this respect, the protection offered by water extracts of the *U.dioica* was comparable to widely used synthetic antioxidant, BHT. In addition, while both pre and post-flowering extracts showed potency in prevention of lipid peroxidation, post-flowering extracts were more potent; so, that can be considered as a suitable alternative for BHT. However, further investigations should be done for finding the ingredients involved in the observed synergistic antioxidant effects.

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