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# Influence of Altitude on Free Radical Scavenging Potential and Total Reducing Capacity of Medicinal Plant Yarrow (*Achillea wilhelmsii* C. Koch)

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**To evaluate the effects of altitude on free radical scavenging potential and total reducing capacity of the medicinal plant *Achillea wilhelmsii* C. Koch, aerial parts of yarrow were collected at the flowering stage from four altitude of Dena (2448, 1890, 1800and 1739 m) and three altitude ofBoyer –Ahmad(2373, 1793 and 1485 m) districts located in Kohgiluyeh and Boyer-Ahmad province (latitude 30.67 N, longitude 51.60E). Free radical scavenging potential and total reducing capacity of methanolic extracts of the plant samples were determined by DPPH (1,1-diphenyl- 2-picrylhydrazylradical) and Folin-Ciocalteu assays, respectively. Free radical scavenging potential expressed as $\mu$mol Trolox equivalent $g^{-1}$ DW ($\mu$mol TE $g^{-1}$ DW) ranged from 508.27 to 155.50. Samples collected from the lowest altitude of Dena and Boyer-Ahmad-districts (samples "D" and "G") showed the highest free radical scavenging potential. Total reducing capacity ranged from 65.15 to 51.83 mg gallic acid equivalent $g^{-1}$ DW (mg GAE $g^{-1}$ DW). Samples collected from the lowest altitude of Boyer-Ahmad district (Samples "G") showed the highest total reducing capacity with 22.6 percent increase compared to the highest altitude in this region. Although free radical scavenging potential and total reducing capacity both increased with reduced altitude in Boyer-Ahmad, such close relationship was not observed in Dena district. It is concluded that free radical scavenging potential and total reducing capacity of the medicinal plant yarrow is affected by altitude and lower altitude may favor higher antioxidant capacity.**

**Key words**: *Achillea wilhelmsii*, altitude, DPPH, Folin-Ciocalteu, medicinal plant

Molecular oxygen acts mainly as final electron acceptor during cellular respiration. However, as a result of partial reduction reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide are formed (Apel and Hirt 2004; Arora *et al*., 2002). There is a balance between production and removed of ROS in the cell. The scavenging of ROS is performed by the antioxidant enzymes such as catalase, superoxide dismutase and ascorbate peroxidase and by the low molecular weight compounds such as carotenoids and plant phenolics (Karuppanapandian *et al*., 2011). Under stress condition and due to the environmental pollutants, production of ROS overrides its removed by the antioxidant systems, causing oxidative stress in the cell (Tiwari 2004). Oxidative stress may cause various diseases such as atherosclerosis, cancer and diabetes (Halliwell *et al*., 1992). Supplementary antioxidant may prevent many of the disorders caused by oxidative stress (Havsteen 2002). Due to probable harmful effects of synthetic antioxidant, various sources of natural antioxidant are suggested by investigators (Ito *et al*., 1983; Frankel 2001; Rice-Evans *et al*., 1995; Okuda 1999). Many plants, medicinal plants in particular, are rich in

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antioxidant such as carotenoids vitamin C, vitamin E and polyphenols that can scavenge ROS and reduce oxidative stress in the cell (Rice-Evans 2004; Burk *et al*., 2010; Panovska *et al*., 2005). Asteraceae (Compositae) is the largest family of flowering plants with about 900 genera and more than 12000 species (Ozgen *et al*., 2004). The genus *Achillea* is represented by about 85 species most of which are distributed in Europe and Asia (Konemann 1999). Several pharmacological properties of this genus such as antioxidant (Candan *et al*., 2003), antihyperlipidemia (Asgary *et al*. 2000), antitumor (Tozyo *et al*., 1994; Csupor-Loffler *et al*., 2009), antimicrobial (Stojanovic *et al*. 2005) and hepatoprotective activities (Yaeesh *et al*., 2006) have been reported.*Achillea wilhelmsii* C.Koch is a perennial medicinal herb which has relatively wide distribution in different parts of Iran (Asgary *et al*. 2000). It contains a wide variety of secondary plant products including flavonoids and sesquiterpenelactones (Asgary *et al*., 2000). Although the essential oil chemical composition and the antioxidant potential of *Achillea wilhelmsii* C. Koch have been under intensive investigation (Javidnia *et al*., 2004; Afshrypour *et al*., 1996; Shahraki and Ravandeh 2012; Mahsan *et al*., 2014), to the best of our knowledge, reports on the effects of altitude on the essential oil constituents, antioxidant potential and total reducing capacity of this medicinal plant are scare. The quantity and quality of the essential oil in the aerial parts of the perennial medicinal plant *Tanacetum polycephalum* were shown to be affected by altitude (Mahdavi *et al*., 2013). In another medicinal plant *Stachysinflata*, essential oil composition and quantity was influenced by the altitude at which plants grow(Shabazi *et al*., 2014). In *Artemisia roxburghiana*, the oil yield was lowest in the plants collected from higher altitude (Haider *et al*., 2009). In addition, the essential oil constituents and percentages of this plant were affected by altitude. In a previous study we demonstrated the effects of altitude on the essential oil constituents of *Achillea wilhelmsii* C.Koch (Yazdanpanah *et al*., 2014). In the present work the influence of altitude on the free radical scavenging potential and total reducing capacity of this perennial medicinal plant is reported and compared with the data in the literature.

## MATERIALS AND METHODS

**Plant material:**

The aerial parts of *Achillea wilhelmsii* C.Koch, at the flowering stage, were collected during the summer of 2013 from seven locations of Kohgiluyeh and Boyer–Ahmad province as illustrated in Table 1. Identification of the species was performed the botanist at the biology department, Shiraz University.

**Preparation of the methanolic extracts**

The collected samples were air-dried at room temperature in the dark. One gram of each finely ground sample was extracted with 30 ml methanol for 12 hrs at room temperature with continues shaking. The mixture was then centrifuged at 4000 rpm and the supernatant was used for free radical scavenging potential and total reducing capacity determinations.

**Measurement of free radical scavenging potential by DPPH assay**

Measurement of radical scavenging capacity by DPPH assay was performed as described by Thaipong *et al*., (2006). In brief, 150 µL of standard solution or sample was added to 2850 µL DPPH solution and kept in the dark. After 60 min the absorbance of the solution was measured at 515 nm. Trolox in the concentration rang of 25 to 800 µM in methanol was used for calibration curve construction. Free radical scavenging potential is reported as µmol trolox equivalent per gram dry weight (µmol TE $g^{-1}$ DW). The percent inhibition of the DPPH radical by one ml of the extract was calculated according to the following equation:

$$\%\ \text{inhibition} = \left(\left(\frac{A_c - A_s}{A_c}\right)\right) \times 100$$

Ac is the absorbance of control at 515 nm and As is the absorbance of sample after 60 min incubation. The concentration of methanolic extract scavenging 50% of the DPPH radicals ($IC_{50}$) was calculated from the plot of the DPPH absorbance at 515 nm versus extract concentrations.

**Determination of total reducing capacity**

Folin-Ciocalteu assay was performed as described by Velioglu *et al*., (1998). In brief, 200 µL of standard solution or sample was mixed with 1.5ml of Folin-Ciocalteu reagent which was previously diluted ten-fold with distilled water. After 5 min,

1.5ml of 6% (w/v) sodium bicarbonate solution was added to the solution. The mixture was kept for 90 min at room temperature and the absorbance was recorded at 750 nm. Gallic acid was used as standard in the concentration range of 25-200 µg $ml^{-1}$.

**Statistical analysis**

Each experiment had three replicates and data are shown as mean ± SE. Means were compared using SPSS 16.0. Duncan's multiple range test was used to determine significant differences at $p<0.05$.

## RESULTS AND DISCUSSION

**Free radical scavenging potential**

The plots of DPPH radical absorbance at 515 nm versus extracts concentrations were linear for all the extracts and are shown for the samples with the lowest and the highest radical scavenging potentials (samples "E" and "G", respectively) in Fig. 1. With samples "G", 20 mg $ml^{-1}$ of the extract scavenged nearly all the DPPH radical and therefore reduced absorbance at 515 nm from about 1.0 to near zero. In contrast, 20 mg $ml^{-1}$ of sample "E" only reduced absorbance at 515 nm from 1.1 to about 0.6. In Boyer-Ahmad district, the free radical scavenging potential, expressed as µmol Trolox equivalent $g^{-1}$ DW, increased with decrease in altitude (samples "E", "F" and "G" in Table 2). In Dena district, although the highest scavenging potential was observed at the lowest altitude (Sample "D"), the scavenging potential was not inversely related to the altitude. As shown in Table 2, percent inhibition of DPPH radical followed the same order as the free radical scavenging potential and ranged from 58.76 to 18.33 percent. $IC_{50}$, expressed as mg $ml^{-1}$, ranged between 9.64 to 28.3 for samples "G" and "E", respectively. It is clear that samples with higher free radical scavenging potential have lower $IC_{50}$.

**Table 1.** Districts, locations and altitudes of Kohgiluyeh and Boyer-Ahmad province at which plant samples were collected

| Districts | locations | Altitudes(m) |
|---|---|---|
| | A)Padena | 2448 |
| Dena | B)Amirabad | 1890 |
| | C)Bhrambegi | 1800 |
| | D)Kara | 1739 |
| | E)Galal | 2373 |
| Boyer-Ahmad | F)Thlion | 1793 |
| | G)Chin | 1485 |

**Total reducing capacity**

The plots of absorbance at 750 nm versus extracts concentrations were linear for all the samples and are shown for the samples "E" and "G" in Fig. 2. The increase in absorbance at 750 nm is due to probable reduction of Mo (VI) to Mo (V) in Folin-Ciocateu reagent by the reducing substances present in the extracts. With samples "E" and "G", 30 mg ml-1 of the extracts increased absorbance at 750 nm from about 0.04 to about 0.85 and 1.1, respectively. This indicates that samples "G" contained more reducing substances, such as plant phenolics, compared to sample "E". In Boyer-Ahmad region, as with free radical scavenging potential, total reducing capacity increased with decrease in altitude (Table 2). At

**Table 2.** DPPH radical scavenging potential, percent inhibition, $IC_{50}$ and total reducing capacity of the methanolic extracts *Achillea wilhelmsii* C. Koch aerial parts collected from Denaand Boyer-Ahmad districts in Kohgiluyeh and Boyer-Ahmad

| Districts | Trolox Equivalent (µmol TE g-1 DW) | % inhibition of DPPH radicals | IC50 (mg $ml^{-1}$) | Total reducing capacity (mg GAE g-1 DW) |
|---|---|---|---|---|
| A (2448m) | $382.4\pm7.6^{c}$ | $41.30\pm0.89^{c}$ | $12.91\pm0.06^{c}$ | $57.6 \pm 4.35^{b}$ |
| B (1890m) | $333.2\pm7.9^{d}$ | $39.5\pm0.91^{c}$ | $13.90\pm0.57^{c}$ | $63.90 \pm 3.64^{a}$ |
| C (1800m) | $313.2\pm1.4^{e}$ | $36.53\pm0.18^{d}$ | $15.30\pm0.17^{b}$ | $51.83 \pm 3.52^{c}$ |
| D (1739m) | $412.4\pm1.0^{b}$ | $45.90\pm0.06^{b}$ | $13.30 \pm 0.17^{c}$ | $57.11 \pm 1.90^{b}$ |
| E (2373m) | $155.5\pm1.4^{f}$ | $18.33\pm0.14^{e}$ | $26.30 \pm 1.15^{a}$ | $53.13 \pm 3.75^{bc}$ |
| F (1793m) | $338.3\pm6.6^{d}$ | $36.46\pm0.76^{d}$ | $14.10 \pm 0.51^{bc}$ | $55.73 \pm 4.91^{bc}$ |
| G (1485m) | $508.3\pm4.3^{a}$ | $58.76\pm0.48^{a}$ | $9.64 \pm 0.08^{d}$ | $65.15 \pm 0.50^{a}$ |

province. Values are mean ± SE, and those with different letters are significant different ($p<0.05$).

sites "E" (2373m), "F" (1793m) and "G" (1485m), total reducing capacities were 53.13, 55.73 and 65.15 mg GAE $g^{-1}$ DW, respectively. This inverse relationship was not observed between total reducing capacity and altitude in the Dena district. The lowest value of 51.83 mg GAE $g^{-1}$ Dw was observed at 1800 m (sample "C") whereas the highest value of 63.90 mg GAE $g^{-1}$ DW belonged to the sample "B" at 1890m altitude.

In a previous work, we showed that the essential oil content and oil constituents of *Achillea wilhelmsii* were affected by altitude (Yazdanpanah *et al*., 2014). Although there are ample reports on the antioxidant capacity and phenolic content of the genus *Achillea* (Candan *et al*. 2003; Vitalini *et al*., 2011), studies on the environmental effects, such as growth altitude, on these parameters are scarce. In some wild herbs of the Asteraceae family, such as *Achillea millefolium*, polyphenol caffeoyl derivatives was suggested as one of the main antioxidant compound present in the aerial parts of the tested species (Fraisse *et al*., 2011). (Wojdylo *et al*., 2007) showed that *Achillea millefolium* contains high levels of phenolic acids and flavonoids, such as caffeic acid and luteolin, with high antioxidant capacity as measured by DPPH, FRAP and ABTS assays. Positive and significant correlation was observed between the antioxidant capacity and total phenolics of some selected herbs, suggesting that phenolics were the dominant antioxidant compounds. Evaluation of the antioxidant and antiplasmodial activities of the isolated pure compound from methanolic extract of *Achillea millefolium* suggested that some flavonoids glycosides such as luteolin 7-O-glycoside and apigenin 42 -O-glucoside were the main components responsible for both investigated activities (Vitalini *et al*., 2011). In the present study, in the Boyer-Ahmad district both free radical

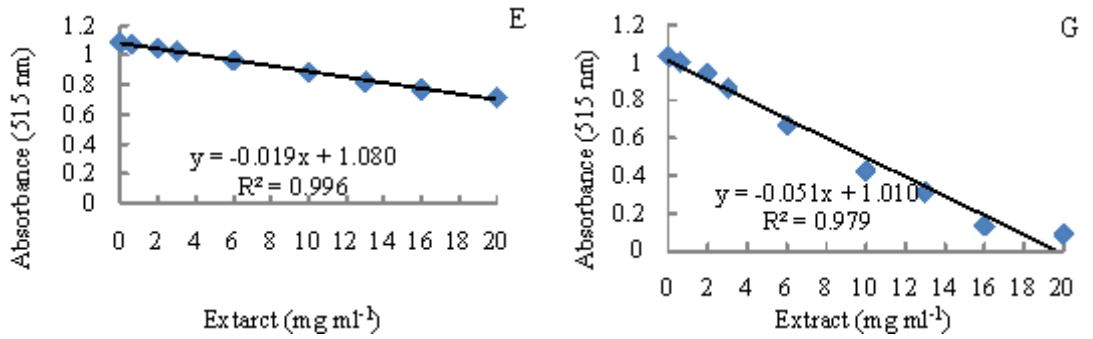


**Fig. 1.** Theeffects of extracts concentrations on DPPH radical absorbance at 515 nm.Samples were collected from Boyer-Ahmad district at 2373 (samples "E")and 1485m (sample "G") altitudes.

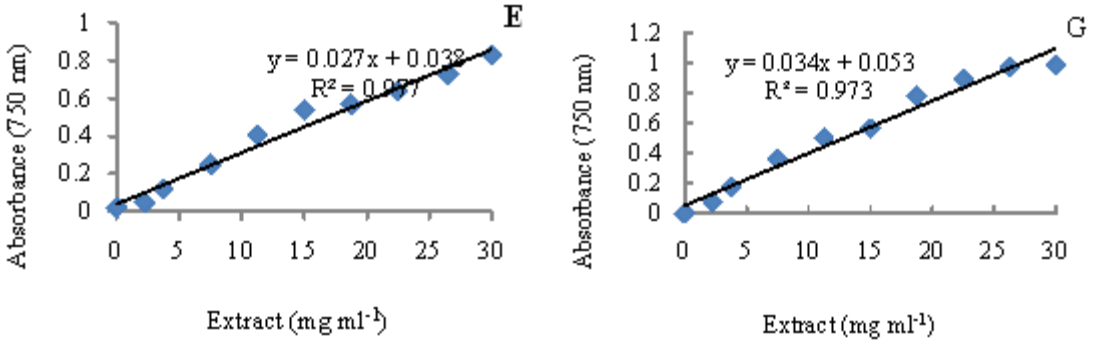


**Fig.2**.The effect of different extracts concentrations on absorbance at 750 nm. Increase in absorbance is due to the suggested reduction of Mo (VI) to Mo (V) in Folin-Ciocalteu regent. Samples were collected from Boyer-Ahmad region at altitude of 2373 m (samples "E") and 1485 m (samples "G").

scavenging potential and total reducing capacity increased with reduced altitude. This suggests that reducing substances, such as phenolic acids and flavonoids, are partly responsible for antioxidant potential of *Achillea wilhelmsii*. Since such a relationship was not observed in Dena district, it may be concluded that other compounds that concentrations are seemingly not much influenced by altitude and are also not detected by Folin-Ciocalteu assay have significant contribution to antioxidant potential of *Achillea wilhelmsii*. Significant positive correlations between altitude and flavonoidrutin or total phenolic acid content were reported for two tartary buckwheat varieties ($p<0.05$) (Guo *et al*., 2011). Since no significant correlation was found between antioxidant property measured by DPPH and ABTS assays and total phenolic content ($p<0.05$) it was suggested that phenolic compounds were not the only components inferring antioxidant property to buckwheat. In another study, methanolic and acetonic extracts of tartarbuck wheat collected from Western Himalaya showed significant increase in both total polyphenols content and antioxidant potential with rising altitude (Kishore *et al*., 2010). In flowering heads of *Arnica montana* grown at altitudes between 590 and 2230 m in Austria, flavonoids, the total amount of caffeic acid derivatives and radical scavenging potential of extract increased with elevation (Spitaler *et al*., 2006). In *Rosa damascena* collected from different altitudes, higher antioxidant activity was observed at lower altitude (Baziar *et al*., 2013). Gallic acid content of samples was maximum at the lowest altitude of 1540 m above sea level. Anthocyanins content of *Sambucus nigra* and *Vaccinium myrtillus* decreased with rising altitude (Rieger *et al*., 2008). In general, decrease in temperature and enhanced solar UV-B radiation with rise in altitude could trigger augmented biosynthesis of antioxidant phenolics, such as flavonoids, in higher plants (Guo *et al*., 2011). Among multitude of environmental factors, the impacts of increased UV-B radiation and reduced temperature with rise in altitude on secondary plant metabolites have been thoroughly discussed (Spitaler *et al*.,2006; Guo *et al*., 2011; Sptaler *et al*., 2008). These authors showed that the contents of sesquiterpene lactones and flavonoids were not positively correlated with the altitudes at which plants grew. However, the relative amount of flavonoids with vicinal-free hydroxyl group in ring B to flavonoids lacking this character increased significantly with elevation.Correlations between total phenolics, antioxidant capacity and changes in altitude reported by various investigators are not similar. It seems that, in addition to genetic makeup of the species and cultivars, structural differences in phenolics compounds and terpenoids and their derivatives affect the correlations between secondary plant products and altitude.

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