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# Evaluation of *Achillea millefolium L.* Biochemical Changes in Iran's Natural Habitat

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***Achillea millefolium L.*** **is one of the most important medicinal plants with antioxidant compounds. The use of compounds derived from plants reduces the incidence of many chronic diseases. The purpose of this investigation is study of total phenolic content and antioxidant activity some of ecotypes yarrow grown in natural habitats in Iran. This experimental study was conducted in 2013 at the Islamic Azad University, Tonekabon Branch. After identifying the natural sites, we have attempted to harvest of aerial part and after drying in lab temperature, essential oil was extracted by steam distillation. In this research for evaluate the antioxidant properties was used of three method, DPPH, Antioxidant capacity ferro revival and phosphomolybdenum, that all mechanism is based on the electron donating. All ecotypes had antioxidant activity and ecotypes grown in Kandovan region were measured with the most total phenolic (89.5 mg GA/g dew) and flavonoid (20.4 $\mu$g/g dew) and the lowest in Saveh (71.3 mg GA/g dew, 17.4 $\mu$g/g dew). Variation of the antioxidant properties were significant (*Pd"0.01*) in areas and were accounted Kandovan with highest value and the lowest in Save. As a result, yarrow essential oil grown in Kandovan in terms of amount of total phenolic, flavonoid and antioxidant property, it was determined the best natural habitat.**

**Key word:** *Achillea millefolium L.*, Antioxidant Compounds, DPPH, Total Phenolic, Flavonoid Natural Habitats.

There is plenty of evidence to indicate toxicity and nutritional effects of synthetic antioxidants added to foods such as butylated hydroxyl anisole, butylated hydroxyl- toluene, tetra beta hydroxyl quinine and order (Ito *et al.,* 1983). In addition to the risk of liver damage and cancer causing in laboratory animals has the disadvantages of using synthetic antioxidants (Nemeth, 2008). Hence the need for less toxic and more effective strong antioxidants is a serious necessity (Garcia *et al.,* 1997). That is why many leading nutritionists today antioxidants needed to supply the body are recommended with eating herbs, fruits and vegetables, because they typically consume plant antioxidants are better fewer side effects and the treatment (Frankel, 2001). Considering that plants are important sources of antioxidants, studies are increasing in this field. These plants with rich antioxidant compounds, can cause are protected from oxidative damage to the cells (Rice-Evans, 2004). Natural antioxidants increases plasma antioxidant power and reduce risk of some diseases, such as cancer, heart disease and stroke (Prior *et al.,* 2000). Medicinal plants are God-given blessings that are valuable legacy for the health of human society according to statistics the World Health Organization, about 80 percent of the world population for primary health care prefers to use plant extracts or their active substances (Rojhan, 2004). Lower side-effects of herbal medicines and

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various combinations of them has cause despite of the presence of drugs of chemical origin, medicinal plants are important and special feature (Benedek *et al.,* 2007). Therefore to domesticate of species of medicinal value and culture at extensive, not only reduces the pressure exerted on natural areas, but also provides the basis for mass production and thus meet the needs of the plants and even pharmaceutical products export (Omidbeigy, 2001).

There are about 100 species of the genus *Achillea* is that most are scattered in Europe, Asia and parts of North America (Candan *et al*., 2003). Species of yarrow (*Achillea millefolium subsp. Millefolium*) one of the species with medicinal and industrial valuable of this plant family is found in pastures (Haidara *et al*., 2006). Yarrow herbs have anti-inflammatory properties and are used for treat wounds and burns (David *et al,* 2010). This plant grows in areas of Iran and is one of the important medicinal plants used to in traditional medicine (Afsharpuor, 1996). Considering that are involved climatic factors such as climate, soil, height growth differences in the various species, extraction techniques and methods to measure the amount of antioxidants in plant secondary metabolites such as phenol, total flavonoid and antioxidant properties (Benetis *et al.,* 2008). So this study was conducted to evaluate the antioxidant activity and phenolic compounds yarrow grown in different areas in Iran for therapeutic applications and further research.

## MATERIALS AND METHODS

The aerial part of *A. millefolium* (L.), growing wild in different regions of Iran (Table 1), was collected in May 2013. At each height, were collected branches and leaves during a 90-meter transect with distance of 15 meters, from 6 points in randomly. Voucher specimens were deposited in the herbarium of the Department of Biology, Islamic Azad University of Tonekabon, Iran. The plants were dried at room temperature. The essential oil yield of *A. millefolium* (L.) was 0.23% (v/w) that was extracted with steam distillation for 4h of 100g of air dried plants. All determinations were performed in triplicate (n = 3).

**Estimation of total polyphenol content**

Total polyphenol content was measured using Folin–Ciocalteu colorimetric method described previously by Gao *et al,*. 2000. The total polyphenol levels in samples extracted was calculated using a standard curve based on mg GA/g dry extract weight.

**Determination of total flavonoid content**

Total flavonoid content was determined using Zhishen *et al.,* (1999) method. The total flavonoid levels in samples extracted was calculated using a standard curve based on ¼g / g dry extract weight.

**Free radical-scavenging ability by the use of a stable DPPH radical**

The DPPH radical-scavenging activity was determined using the method proposed by Re *et al.,* (1999).The first was calculated percentage inhibition of free radical. Then antioxidant activity was expressed of the extract samples using the standard curve based on ¼mol T / g dry extract weight. Trolex structure similar to that of vitamin E and used as standard in a wide range of research (Re *et al.,* 1999).

**Determination of the Ferric Reducing Ability**

The ferric reducing ability was determined using the method proposed by Benzie *et al.,* 1996. The ferric reducing ability of the extract samples using a standard curve based on µmol Fe/mg dry extract weight.

**Determination of the Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex**

The antioxidant capacity of a phosphomolybdenum complex was determined using the method proposed by Prieto *et al.,* 1999. Then antioxidant activity was expressed of the extract samples using a standard curve based on µmol T/mg dry extract weight.

**Statistical analysis**

Obtained data was analyzed by correlation and completely random design, using SPSS software version 13 with the level of statistically significant $P<0.05$.

## RESULT AND DISCUSSION

The total phenolic content and antioxidant properties of a species in each area are depended on many parameters such as climate, soil, elevation and various subspecies (David *et al.,* 2010). Therefore the purpose of this research, are studied of total phenols, flavonoid and antioxidant activities

of *A. millefolium* (L.) grown in different regions of Iran. Comparisons of mean and standard deviation of variables in samples taken from different areas showed that there were significant differences between the parameters of and genotypes in different regions are with diverse potentials in the production of phenolic compounds and other antioxidants (Table 2).

Correlation table (Table 3) showed significant relationship between the variables at 5% and 1% which reflects changes of impressible phenolic substance is with other antioxidants. The results of total phenol in samples collected from different regions indicated that the highest amount in the Kandovan and the lowest values were measured in regions of Damash and Golestan (Fig. 1 and Table 1). Phenolic content of the samples was calculated in the range of 71-89 mg. Analysis of total flavonoid in samples collected measured data showed the highest total flavonoid content in Kandovan and the lowest values in regions of Damash and Saveh (Fig. 2 and Table 1).

The changes of DPPH in this research indicated that these changes were significant regions of in the region Kandovan with largest and lowest values were measured in Saveh region (Fig. 3 - Table 1). The range of this radical trapping activity of the extracts was measured among 473-523 (µmol T/g dew).

In measuring of antioxidant activity of ferro revived, its range among 462 to 408 µmol Fe/mg dew was obtained with maximum value in Kandovan and minimum value In the Ramsar and Damash (Fig. 4 and Table 1).

In the method of phosphomolybdenum was calculated in the range of antioxidant activity ranged from 482 to 572 µmol T/mg dew with highest value in Kandovan and the lowest in Saveh (Fig. 5 and Table 1).

Dendrogram of biochemical analyzes (Fig.6) showed that the reactions of genotypes were varied in different regions and created in the two groups and two subgroups. A group consists of Polezangooleh, Tonekabon, Siahbishe and the Kandovan and are placed Ramsar, Damash, Saveh and Golestan in B group. The common point these sites, been almost equal height, so that in height below of 2500 m and above of 2800 m, were conclusions identical biochemical reactions for genotypes.

Regression equations between total phenolic and other measured parameters estimated significant relationships with high regression coefficients that can be expressed as the linear equation (Fig. 7, 8, 9, and 10).

In a disquisition, amount of total phenolic in ethanol extract was measured 118 mg GA/g dew (David *et al.,* 2010) that is more than the amount of calculated in this research. These differences may be caused by using different methods of measurement, extraction, different standards and different climatic conditions. The main compounds forming of yarrow are the essential oils and flavonoids. The flavonoids in yarrow such as quercetin and rutin cause anti-spasmodic activity (Nakayoma *et al.,* 1995). Therapeutic properties yarrow is due to its anti-radical. The results also showed that the yarrow has anti-radical properties that are consistent with some studies (Nickavar *et al.,* 2006). The experiment mechanism of antioxidant activity of ferro revived it is based on ability of regenerative in yellow complexes at tripyridine triazyl ferric to the blue ferrous complex, that are carried by the electron donors of antioxidants (Benzie *et al.,* 1996). The blue can be measured by spectrophotometer at a wavelength of

**Table 1.** Collection sites of the 48 populations

| Sample Number | Altitude | Province |
|---|---|---|
| $K_1 \ldots K_6$ | 3015m | Kandovan, Alborz, Iran |
| $Pz_1 \ldots Pz_6$ | 2827m | Pole-zangoole, Alborz, Iran |
| $S_1 \ldots S_6$ | 2954m | Siahbishe, Alborz, Iran |
| $Sa_1 \ldots Sa_6$ | 1680 m | Saveh, Markazi, Iran |
| $R_1 \ldots R_6$ | 2470 m | Ramsar,Mazandaran, Iran |
| $T_1 \ldots T_6$ | 2900 m | Tonekabon, Mazandaran, Iran |
| $D_1 \ldots D_6$ | 1712 m | Damash, Guilan,Iran |
| $G_1 \ldots G_3$ | 1720 m | Golestan,Iran |

**Table 2.** Comparisons of mean and standard deviation of variables in samples taken from different areas

| Variables / Provinces | M ± SD | | | | |
|---|---|---|---|---|---|
| | Total Phenol (mg GA/g dew) | Total Flavonoid (µg/g dew) | DPPH (µmol T/g dew) | Anti Oxidant Ferro (µmol Fe/mg dew) | Anti Oxidant Mo (µmol T/mg dew) |
| Saveh-1680 m | $71.3533^{g}$ ±0.59341 | $17.4867^{c}$±0.42016 | $473.00^{e}$±3.000 | $408.67^{e}$±4.509 | $482.67^{f}$±5.033 |
| Golestan-1720 m | $77.1667^{f}$±1.04083 | $18.8333^{b}$±0.45092 | $497.00^{c}$±2.646 | $426.67^{d}$±7.767 | $521.00^{d}$±5.000 |
| Kandevan-3015 m | $89.5333^{a}$±1.33167 | $20.4333^{a}$±0.81445 | $532.67^{a}$±4.041 | $462.00^{a}$±5.568 | $572.67^{a}$±4.041 |
| Polezangoole-2827 m | $82.3000^{c}$±0.62450 | $19.0133^{b}$±0.01528 | $511.67^{b}$±4.021 | $438.67^{c}$±3.055 | $541.33^{c}$±4.509 |
| Siahbishe-2954 m | $85.9000^{b}$±0.20000 | $20.0600^{a}$±0.25060 | $519.33^{b}$±5.508 | $450.67^{b}$±4.726 | $558.67^{b}$±4.041 |
| Tonekabon-2900 m | $84.3333^{c}$±0.51316 | $19.3000^{b}$±0.40000 | $515.33^{b}$±7.024 | $441.00^{c}$±4.583 | $548.67^{c}$±5.508 |
| Ramsar-2470 m | $79.4667^{e}$±0.50332 | $19.0000^{b}$±0.20000 | $489.33^{cd}$±4.509 | $421.67^{d}$±5.033 | $513.33^{d}$±5.508 |
| Damash-1712 m | $77.4333^{f}$±1.50444 | $18.1000^{c}$±0.10000 | $483.67^{d}$±5.508 | $418.67^{d}$±3.055 | $499.00^{e}$±2.000 |
| $F_s$ | 124.146** | 16.644* | 54.975* | 38.134* | 136.295* |

*, **Significant at the 5% and 1% probability levels respectively.

593 nm and are examined linear relationship with the power of antioxidants and electron donors (Huang *et al.,* 2005). Phosphomolybdenum method is one of the spectrometry technique a through which to complex formation phosphomolybdenum measured antioxidant potential (Kanner *et al.,* 1994). The revival of ferric is used for as an index




**Fig. 1.** Variations in amount of total phenols content in samples of different areas




**Fig. 2.** Variations in amount of flavonoid content in samples of different areas




**Fig. 3.** Variations in amount of DPPH content in samples of different areas

**Table 3.** Correlation coefficient amount various traits.

| Variables | Anti Oxidant Mo (µmol T/mg dew) | Anti Oxidant Ferro (µmol Fe/mg dew) | DPPH (µmol T/g dew) | Total Flavonoid (µg/g dew) |
|---|---|---|---|---|
| Total Phenol(mg GA/g dew) | .863* | .853* | .841* | .923** |
| Total Flavonoid(µg/g dew) | .927** | .933** | .807* | 1 |
| DPPH(µmol T/g dew) | .887* | .975** | 1 | - |
| Anti Oxidant Ferro(µmol Fe/mg dew) | .881* | 1 | - | - |
| Anti Oxidant Mo(µmol T/mg dew) | 1 | - | - | - |

*, **Significant at the 5% and 1% probability levels respectively. Mo: Molybdenum

of electrons potential. This method is based on the mechanism of the increase in absorbance of the reaction mixture. In this method, antioxidant compounds are polymerized with potassium ferrocyanide, trichloride ascetic and ferric chloride. The obtained green complex was measured at a wavelength of 700 nm. The increase in absorbance of the reaction mixture has implications for the power typical renewal (Jayaprakash *et al.,* 2001).

Based on results of this research, all phenolic compounds in collected yarrow genotypes has acted as an electron donor and may be able ended unwanted reactions caused by free radicals in the human body. Finally, this study showed that

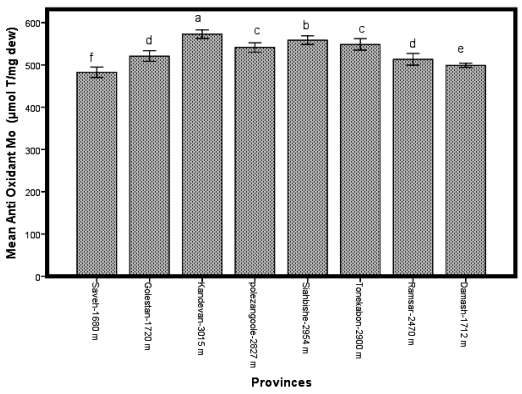


**Fig. 4.** Variations in amount of antioxidant activity based ferro revived (µmol Fe/mg dew) content in samples of different areas

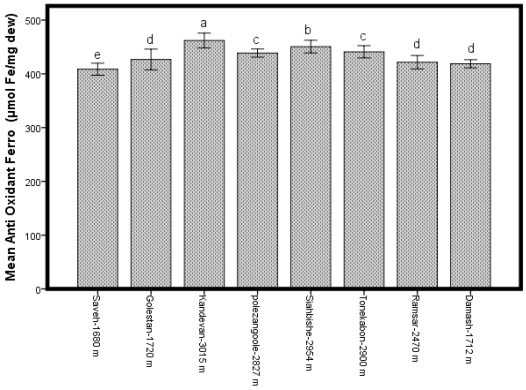


**Fig. 5.** Variations in amount of antioxidant activity based phosphomolybdenum (µmol T/mg dew) content in samples of different areas.

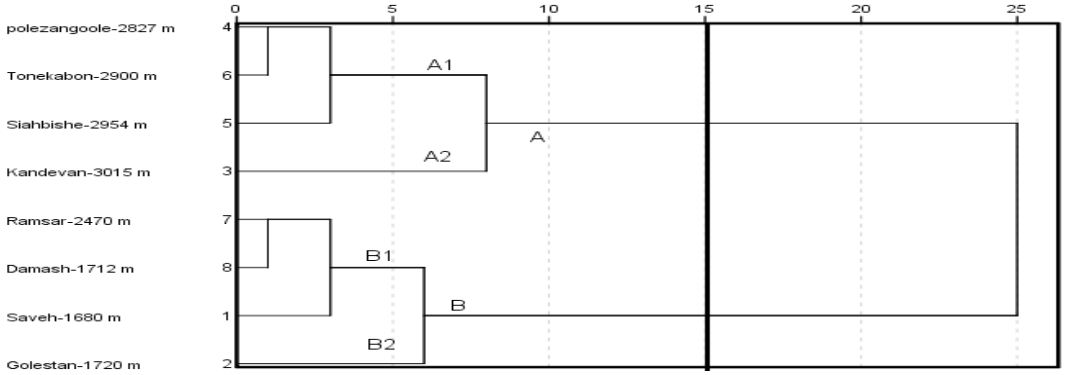


**Fig. 6.** Dendrogram obtained by cluster analysis based on biochemical analyzes

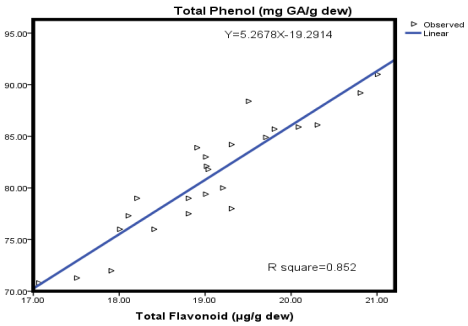


**Fig. 7.** The regression curve of total phenolic and flavonoid

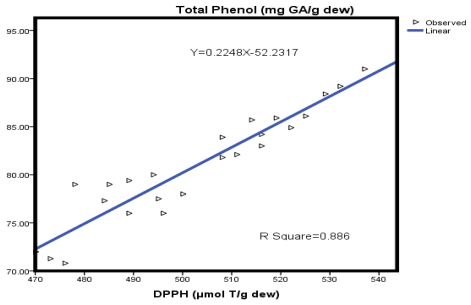


**Fig. 8.** The regression curve of total phenolic and DPPH

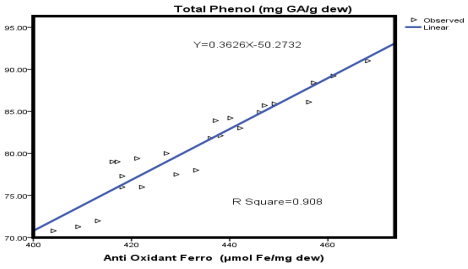


**Fig. 9.** The regression curve of total phenolic and anti oxidant ferro

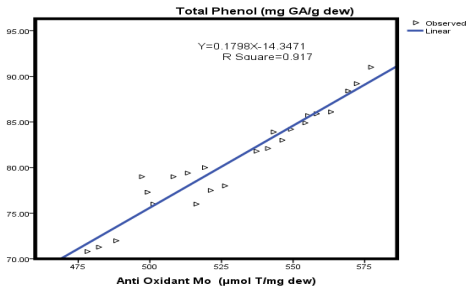


**Fig. 10.** The regression curve of total phenolic and anti oxidant Mo

extracts of yarrow genotypes in all the models studied have different levels of antioxidant activity. This difference could be related to genetic and environmental causes. So they can be a source of promising for the supply of natural resources antioxidant.

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