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# The effect of environmental pollutions on phytochemical parameters and antioxidant activity of *Quince, Apple*, and *Mulberry* fruits

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**Key words:** Antioxidant activity, Environmental pollution, Flavonoid, Phenoilc compounds.

**Abstract**

Human actions are causing the slow killing of plant and animal species in nature through toxic pollution due to industrial and technological advancement in recent decades. To reveal metal toxicity, the effects of industrial pollution on phytochemical parameters of *Malus domestica*, *Cydonia oblonga* and *Morus alba* were determined. So, plants were collected from east of Isfahan area (surrounding industrial areas: pollution area and non-pollution area), Iran and the contents of phenolic compounds, total flavonoid and antioxidant activity were investigated. Survey results indicated that the amount of phenol, flavonoid and antioxidant activity were decreased in all polluted samples but a non-significant decrease was observed in *Cydonia oblonga*. These results showed that different response in polluted plants and non-polluted plants is due to induction oxidative stress and environmental toxicity in plants.

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## Introduction

Heavy metals have been extremely released into the environment due to rapid industrialization and have created a major world worry. Heavy metals are often detected in industrial wastewaters, which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, printing and photographic industries, etc. In contrast to organic wastes, heavy metals are non-biodegradable and they can be accumulated in living tissues, causing different diseases and disorders (Kadirvelu *et al.,* 2001a; Williams *et al.,* 1998).

Intake and accumulation of heavy metals in vegetables and fruits are determined by many factors, including: concentration of heavy metals in soil, composition and intensity of atmospheric deposition, including rainfall, phase of plant vegetation (Vontsa *et al.,* 1996).

The absorption of heavy metals can lead to altering of humans and animals healthiness state. Then, the carcinogenic effects generated by continuous utilization of fruits and vegetables loaded with heavy metals (Turkdogan *et al.,* 2002).

Heavy metals have been especially produced Reactive oxygen species (ROS). They are among the main sources of primary catalysts that initiate oxidation in vivo and in vitro (Wettasinghe and Shahidi, 2000). Oxygen-derived free radicals such as hydroxyl radical and superoxide anion radical are thought to be linked to the onset of different pathological conditions.

A many of natural antioxidants have already been isolated from various kinds of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, roots, spices, and herbs (Ramarathnam *et al.,* 1995). Dietary antioxidants can stimulate cellular defenses and help to inhibit cellular components against oxidative damage (Evans and Halliwell, 2001).

Plants contain a various group of phenolic compounds including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. All the phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants (Reische *et al.,* 1998). Factors inducing the antioxidant activity of plant phenolics include position and degree of hydroxylation, stability of the phenol to food processing operations, polarity, solubility, reducing potential, and stability of the phenolic radical (Decker, 1998).

*Morus alba*, known as white mulberry, is a short-lived, fast-growing, small to medium sized mulberry tree, which grows to 10–20 m tall. The species is native to northern China, and is widely cultivated and naturalized elsewhere (Zhengyi *et al.,* 2013). Apple (*Malus domestica*) belongs to the Rosaceae family. Malus that are used by humans. Apples grow on deciduous trees. Apples have been grown for thousands of years in Asia and Europe, and were brought to North America by European colonists (Pierre-éric *et al.,* 2006). Quince is the fruit of a deciduous tree of the *Rosaceae* family, *Cydonia oblonga* Miller. When ripe, quince fruits impart an agreeable, long-lasting, and powerful flavor (Silva *et al.,* 2000).

Therefore, the present study carried out to evaluate the effect of environmental pollutions on phytochemical parameters and antioxidant activity of *Malus domestica*, *Cydonia oblonga* and *Morus alba* fruits.

## Materials and methods

*Plant materials*

The fruits of *Malus domestica*, *Cydonia oblonga* and *Morus alba* were collected from east of Esfahan area (surrounding industrial areas: pollution area and

non-pollution area), province of Esfahan, Iran, in September 2013. The voucher specimen was deposited at the herbarium of the Research-Institute of Esfahan Forests and Rangelands. The fruits were air-dried under shade and ground in to fine powder using electric blender, then, 20 gr of fruits powder were extracted with 200 ml methanol 80% by Maceration method. In this way, a mixture of methanol and fruit powder were placed on the shaker for 72 hours, then this was smoothed by the filter paper. The residue was evaporated at room temperature and the dried extract was stored at 4°cuntil used. The extract (0.01 gr) was dissolved in distilled water (10 ml) and obtain different concentrations of fruits extract.

*Total phenol determination*

Total phenols were determined by Folin Ciocalteu reagent (Sharafati-Chaleshtori *et al.,* 2011; Sumaya-Martinez *et al.,* 2011). Different concentrations of each plant extract (10, 20, 40, 60, 80, 100 µl) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (1 ml, 1:10 diluted with distilled water) and 7% Na2CO3 (1 ml). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg $L^{-1}$ solutions of gallic acid in methanol : water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg $g^{-1}$ of dry mass), which is a common reference compound.

*Total flavonoid determination*

Aluminum chloride colorimetric method was used for flavonoids determination (Miliauskas *et al.,* 2004; Pourmorad *et al.,* 2006). Each plant extracts (10, 20, 40, 60, 80, 100 µl) in methanol were separately mixed with 1.5 ml of methanol, 1 ml of 2% aluminum chloride, 6 ml of 5% potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 40 min; the absorbance of the reaction mixture was measured at 415 nm for flavonoid assay. The calibration curve was prepared by preparing rutin solutions at concentrations 12.5 to 100 g $ml^{-1}$ in methanol.

*Free radical scavenging activity determination*

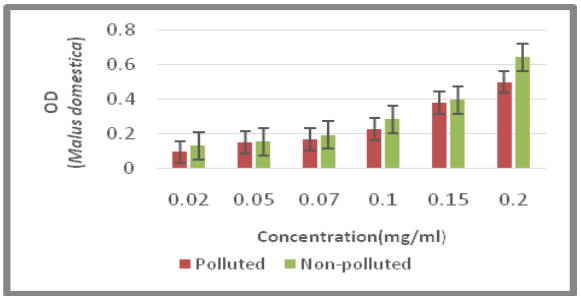
The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Jayaprakasha *et al.,* 2001). Different concentrations of each extract (10, 20, 40, 60, 80, 100 µl) were added, at an equal volume, to methanolic solution of DPPH (0.004g per 100 ml) . After 120 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid were used as standard controls. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

*Statistical analysis*

The experimental design was a split plot in a randomized complete block design with three replications. The presented data included means of three separate experiments ± SD. In order to analyze the data, SPSS software and ANOVA test were used. Thus, the statistical significance between phytochemical activities values of the extracts was evaluated with a LSD test. P values less than 0.05 were considered to be statistically significant.

**Results**

Analysis of data on total phenol showed that fruits of *Malus domestica* and *Morus alba* of accumulated from polluted area showed significant reduction in polyphenol content compared to *Malus domestica* and *Morus alba* of accumulated from non-polluted area and control ($P<0.05$) (Fig. 1), whereas, in fruit of *Cydonia oblonga* of accumulated from polluted area not showed significant changes in polyphenol content compared to *Cydonia oblonga* of accumulated from non-polluted area and control (Fig. 2).



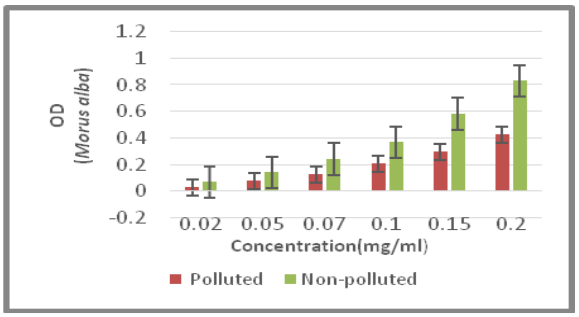



**Fig. 1.** Significant reduction of phenol content (concentration and absorbance) of fruits of accumulated from polluted area in compared with fruits of accumulated from non-polluted area. Bars are least significant differences where $p < 0.05$.

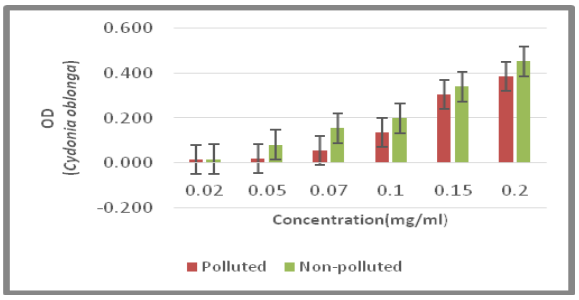



**Fig. 2.** Phenol content (concentration and absorbance) of *Cydonia oblonga* of accumulated from polluted area in compared with *Cydonia oblonga* of accumulated from non-polluted area.

Table 1 show the content of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 29.85x + 0.043$, $r_2 = 0.990$). The total phenol varied from 11.49 ± 0.01 to 26.43 ± 0.02 mg g-1 in the extract powder. Non-polluted *Morus alba* group with total phenol content of 26.43±0.02 mg g-1 had the highest amount among the phenol in this study.

**Table 1.** phenol content in the studied fruits extract.

| Groups | [1]Mean Total phenol ±SD |
|---|---|
| Polluted *Malus domestica* | 11.49±0.01 |
| Non-Polluted *Malus domestica* | 13.73±0.025 |
| Polluted *Morus alba* | 12.73±0.03 |
| Non-Polluted *Morus alba* | 26.43±0.02 |
| Polluted *Cydonia oblonga* | 15.3±0.2 |
| Non-Polluted *Cydonia oblonga* | 20.13±0.02 |

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

When concentration of fruits of *Cydonia oblonga* and *Morus alba* of accumulated from polluted area was increased, the flavonoid content were significantly decreased compared to *Cydonia oblonga* and *Morus alba* of accumulated from non-polluted area and control group ($P<0.05$) (Fig. 3). whereas, in fruit of *Malus domestica* of accumulated from polluted area not showed significant changes in flavonoid content compared to *Malus domestica* of accumulated from non-polluted area and control (Fig. 4).

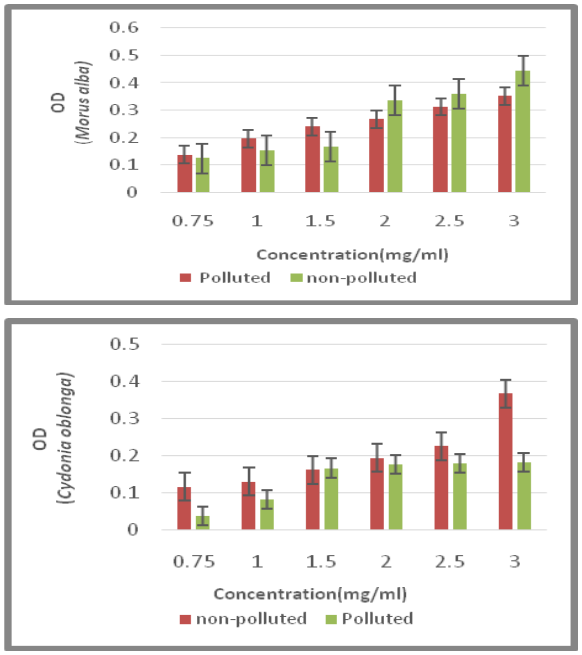



**Fig. 3.** Significant reduction of flavonoid content (concentration and absorbance) of fruits of accumulated from polluted area in compared with fruits of accumulated from non-polluted area. Bars are least significant differences where $p < 0.05$.

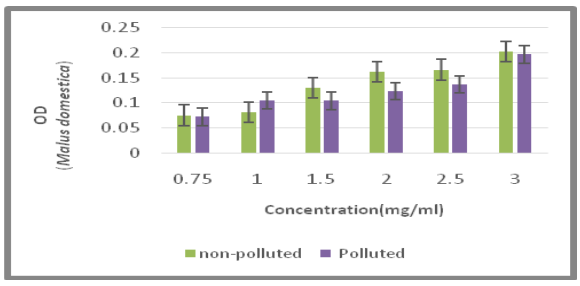



**Fig. 4.** Flavonoid content (concentration and absorbance) of *Malus domestica* of accumulated from polluted area in compared with *Malus domestica* of accumulated from non-polluted area.

The flavonoid content of the extracts in terms of rutin equivalent (the standard curve equation: y = 0.0603x + 0.0007, $r_2$ = 0.985) were between 0.202± 0.002 and 0.49 ± 0.01(Table 2). The flavonoid content in the *Cydonia oblonga* of accumulated from polluted area (0.202 ± 0.002 mg g-1) and in the *Morus alba* of accumulated from polluted area (0.387 ± 0.01 mg g-1) were lower than that in the fruits of non-polluted (0.407 ± 0.003 mg g-1, 0.49± 0.01 mg g-1).

**Table 2.** flavonoid content in the studied fruits extract.

| Groups | [1]Mean Total phenol ±SD |
|---|---|
| Polluted *Malus domestica* | 0.223±0.02 |
| Non-Polluted *Malus domestica* | 0.217±0.01 |
| Polluted *Morus alba* | 0.387±0.01 |
| Non-Polluted *Morus alba* | 0.49±0.01 |
| Polluted *Cydonia oblonga* | 0.202±0.002 |
| Non-Polluted *Cydonia oblonga* | 0.407±0.003 |

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Fig. 5 shows the amount of each extract needed for 50% inhibition (IC50). IC50 of the standard compounds, Ascorbic acid were 103.29 µg/µl. The highest radical scavenging activity was showed by non-polluted Malus with IC50=133.77 which is nearby of Ascorbic acid (Fig. 5).

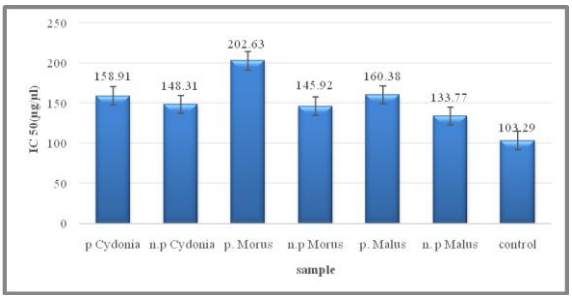



**Fig. 5.** IC50 (µg/µl$^{-1}$) values of fruits for free radical scavenging activity by DPPH radical. Lower IC50 value indicates higher antioxidant activity.

**Discussion**

It has been accepted that flavonoids show antioxidant activity and their effects on human nutrition and health are remarkable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.,* 2003, Cook and Samman, 1996). Phenolic compounds are a class of antioxidant factors which act as free radical terminators (Shahidi and Wanasundara, 1992). Arora *et al.* (2000) show that phenolics (especially flavonoids) are able to change peroxidation kinetics by modifying the lipid packing order. They confirm membranes by decreasing membrane fluidity and prevent the diffusion of free radicals and restrict peroxidative reaction (Arora *et al.,* 2000; Blokhina *et al.,* 2003).

On the other hand, *in vitro* studies have shown that flavonoids can immediately scavenge molecular species of active oxygen: *superoxide*, *hydrogen peroxide*, *hydroxyl radical, singlet oxygen* or *peroxyl radical.* Their antioxidant action resides usually in their ability to donate electrons or hydrogen atoms (Sakihima *et al.,* 2000). Poly-phenols posess perfection structural chemistry for this acitvity and have been shown to be more effective *in vitro* than vitamins E and C on molar basis (Rice-Evins *et al.*, 1997).

The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: y = 0.0603x + 0.0007, $r^2$ = = 0.985) were between 0.202 ± 0.002 and 0.49 ± 0.01 (Table 2). The flavonoid contents in the extracts of non-polluted *M. alba* (0.49 ± 0.01 mg g$^{-1}$) and non-polluted *C. oblonga* (0.407 ± 0.003 mg g$^{-1}$) were higher than that in the extract of *M.domestica*. Table 1 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 29085x + 0.043, $r^2$= 0.9873) . The total phenol varied from 11.49 ± 0.01 to 26.43 ± 0.02 mg g$^{-1}$ in the extract powder. Non-polluted *M. alba* with total phenol contents of 26.43 ± 0.02 mg g$^{-1}$ had the highest amount among the plants in this study. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (Das and Pereira, 1990; Younes, 1981). According to our study, the high contents of these phytochemicals in non-polluted *M.* alba can explain its high radical

scavenging activity.

Free radicals are involved in multitude disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant effect of a special compound or plant extracts (Koleva *et al.,* 2002) . The highest radical scavenging activity was showed by non-polluted *M. domestica* with $IC_{50}$=133.77 µg $µl^{-1}$ which is near that of Ascorbic acid. Therefore, *Cydonia oblonga* was resistant fruit in front of oxidative stress and *Morus alba* fruit was the most sensitive fruit.

**Conclusion**

The result of the present study showed that the high scavenging property of non-polluted *M. alba* may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. All of the extracts in this research exhibited different extent of antioxidant activity. non-polluted *M. alba* extract showed a near potency than Ascorbic acid in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract. Thus, the plants accumulated from industrial area demonstrated that amount of phenol, flavonoid compounds and antioxidant activity significantly were decreased . These changes were related to many factors including metal concentrations, plant species and plant tissues. It has now become clear that high to moderate doses of metal exposure cause generation of free radicals resulting in oxidative damage to final biomolecules, lipids, proteins and DNA.

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