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Comparison of Anti-Oxidant Activities and Exhaustive Extraction Yields between Wild and Cultivated *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* Leaves

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ABSTRACT

Antioxidant activity of natural compounds in food and in dietary supplements plays an important role in healthy life. Scientific evidences suggest that antioxidants reduce the risk for chronic diseases including cancer, diabetes mellitus and heart diseases. The antioxidant activity of wild *Cyclamen persicum, Malva sylvestris* and *Urtica pilulifera* leaves and their cultivated species were studied using 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity and compared to Trolox antioxidant activity. The exhaustive extractions yields for these samples were estimated by using polar and nonpolar solvents. The results showed that the wild *Cyclamen persicum, Malva sylvestris* and *Urtica pilulifera* leaves have higher exhaustive extraction yield and as well the higher antioxidant activity (IC50) comparing with their cultivated species. Both of cultivated, as well the wild natural growing forms of *Cyclamen persicum, Malva sylvestris* and *Urtica pilulifera* are a good source for natural foods supplements, pharmaceutical industry purposes and for organic food rich with antioxidant compounds.

INTRODUCTION

From ancient times, plants have been used for many purposes, including food, medicine, flavoring agents, cosmetics and other uses (Husain *et al.*, 2008). The oxidative damage caused by reactive oxygen species on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart disease, atherosclerosis, cancer and ageing (Finkel and Holbrook, 2000; Madhavi *et al.*, 1996). Fresh organic fruits, vegetables and herbal teas that are rich in natural antioxidant compounds have been associated with the prevention of various types of cancer and cardiovascular diseases (Willcox *et al.*, 2004). Epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age related diseases, such as coronary heart disease and cancer, which may be attributed to their antioxidant activity (Cragg *et al.*, 1997; Eberhardt *et al.*, 2000; Gey, 1990; Kris-Etherton *et al.*, 2002; Willett, 1994). Different studies have suggested that synthetic antioxidants, such as Butylated hydroxyanisole (BHA) butylated hydroxytoluene (BHT), need to be replaced with natural antioxidants, as they were found to be toxic and carcinogenic in tested animal models (Ito *et al.*, 1986; Safer and Al-Nughamish, 1999).

Recently many methods have been developed to evaluate the antioxidant activities of natural plant extracts, numerous in vitro methods have been developed (Mermelstein, 2008). *Cyclamen persicum* Mill.(Persian Cyclamen) belonged to the Primulaceae family is perennial herbaceous plant (Al-Rawi and Chakravarty, 1988). The leaves are oval with heart-shaped base and serrated margins, the length is 3-12cm and width is 2-7cm of dark green color, while the flowers have crown shape various colored of red, white and dark or light pinks (Fig.1) (Michael, 1997).

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In the folk medicine the tubers are crushed and used as a paste to dress on the infected wounds, eczema, psoriases, festering boils and for other skin diseases (Ali-Shtayeh *et al.,* 2000), also used as laxative and antihelmintic (Blasdale, 1954) , whilst the dried leaves have the important roles used in curing the slight skin burns and skin cancer (Oliver-Bever, 1971). The leaves of *Cyclamen persicum* are used as food in Palestine and other Middle East countries to make (Zaamatoot), in which the boiled leaves are filled with rice and condimental minced meat, made into rolls before cooked and eaten with yogurt (Ali-Shtayeh *et al.,* 2008). *Malva sylvestris* L. (Malvaceae family), annual or biennial herbaceous medicinal plant (Barros *et al.,* 2010) usually known as common mallow, is native to Asia, North Africa and Europe. The leaves are green even when dry, have long petioles and are orbicular to reniform, palminervous and lobed (Fig.2) (Gasparetto *et al.,* 2012). Its folk use has been documented since a long-time ago and used as emollient, laxative and anti-cough (Camejo-Rodrigues *et al.,* 2003; Pardo-De-Santayana *et al.,* 2005).

Fig. 1: *Cyclamen persicum* Mill.

Fig. 2: *Malva sylvestris* plant

It is also widely recognized to have anti-inflammatory properties, some other pharmacological and clinical effects are frequently mentioned such as diuretic, laxative, antiseptic, antispasmodic, lenitive, choleretic , bronchodilator, expectorant, antitussive and antiacne activities (Barros *et al.,* 2010; Quave *et al.,* 2008; Zare *et al.,* 2012).Young leaves considered one of the culinary herbs in Palestine and in other Mediterranean countries, they are eaten raw in salads or consumed in soups and as boiled vegetables (Tardio *et al.,* 2006). *Urtica pilulifera* L. (Nettle) is annual herbaceous plant belonging to the plant family Urticaceae. Nettle leaves are dark green color with serrated margins shape (Fig. 3). Fresh nettle could cause blushing and burning of skin when it is touched (Taylor, 2009). The leaves are used to treat stomachache in Turkish folk medicine (Erdemoglu *et al.,* 2003). In addition, this herb is used to treat rheumatism and cough also reduced cold symptoms (Sezik *et al.,* 1997).

Fig. 3: *Urtica pilulifera* plant

It is used for many purposes as medicine, nutrition, fibrous, green color dye, and cosmetic from centuries. Numbers of medical and pharmacologic researches about nettle are increased day by day (Otles and Yalcin, 2012).

Nettle leaves contain anthocyanin glycosides (Kavtaradze and Alaniya, 2003), quercetin, rutin flavonoids (Ji *et al.,* 2007), chlorophylll a, chlorophylll b, β-carotene, and lutein (Sovova *et al.,* 2004).

METHODOLOGY

Materials and reagents

Trolox ((S)-(-)-6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. Methanol analytical grade. All other chemical reagents that are used in the research were purchased from reliable commercial sources.

Instrumentation

The following instrumentations were used: Shaker device (LabTech Shaking Incubator), rotatory evaporator (Heidolph VV2000), heater and stirrer [Heidolph OB2000], Spectrophotometer (Jenway 6505 UV/Vis Spectrophotometer).

Plant material

The Leaves of the wild and cultivated *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* were collected from Jenin region in the West Bank/ Palestine during the spring session (May–June, 2013). Wild plants were collected from the hills and mountains, while cultivated plants collected from the green house from Jenin region. Leaves of the plants species under

study were washed twice with triple distilled water, dried in an oven at an average temperature of 40 °C, for 72 hours and stored in a dry place. The herbariums of plant material prepared and further identified by the pharmacognosist Dr. Nidal Jaradat at the Pharmacognosy laboratory, department of Pharmacy, An-Najah National University and found that the voucher specimen number for *Cyclamen persicum* is (Pharm-PCT-777), *Malva sylvestris* voucher specimen number is (Pharm-PCT-1507) and *Urtica pilulifera* voucher specimen number is (Pharm-PCT-2561)

Preparing of plants extracts

A. For evaluating of the antioxidant capacity of studied plants, the plants leaves were powdered separately using a grinder. The extraction was performed at room temperature. About 100g of the powdered leaves were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per minute for 72 hours and stored in refrigerator for 4 days. The extracts were then filtered using filter papers. The extract was then concentrated under vacuum on a rotatory evaporator. The crude extract was stored at 4ºC for further use. **B.** For evaluating the plants exhaustive extraction yields, 25 gram of the plant dried powder soaked in mixtures of 150 ml 50% ethanol and 50 ml of hexane in well closed Erlenmeyer flask. Then the containers placed in the shaking incubator for 72 hours of 200 round/min shaking at 25ºC, after that the soaked materials were filtered by using semi permeable filter paper and suction vacuum (the filtration is done by Buchner funnel and white man paper No-1 at room temperature). The organic phase and the aqueous phase extracted from each other by using a separator funnel. One hundred and fifty ml of 50% ethanol was added to the same powdered sample. The extraction was repeated and placed in the ''shaker'' for further extraction for another 72 hour then the procedure repeated as the first extraction (Aruna *et al.,* 2012; Rojas *et al.,* 2006). The organic phase evaporated under the hood and then weighted and the aqueous phase evaporated in a rotator evaporator for one hour at 35ºC, to get rid of ethanol after that the aqueous phase freeze dried and weighted the yield. These procedures repeated for the six samples.

Anti oxidant activity

Trolox standard and plant working solutions

A stock solution of a concentration of 1mg/1ml in methanol was firstly prepared for all samples of plant extracts and the standard trolox. The working solutions of the following concentrations (1, 2,3,5,7,10,20,30,40,50,80,100µg/ml) were prepared by suitable dilution with methanol from the stock solution.

Spectrophotometric measurements

2, 2-diphenylpicrylhydrazyl (DPPH) was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above prepared working concentration in a ratio of 1:1:1 respectively. The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 minute at room temperature before the absorbance readings were recorded at 517nm.

Percentage of inhibition of DPPH activity

The percentage of antioxidant activity of the plants and the Trolox standard were calculated using the following formula: The DPPH radical scavenging activity (S %) was calculated using the following equation:

$$S\% = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} is the absorbance of the test sample. The antioxidant half maximal inhibitory concentration (IC_{50}) for the plant samples and the standard were calculated using BioDataFit edition 1.2 (data fit for biologist).

Data analysis

The antioxidant activity was reported as a percentage of DPPH reduction. The inhibition of the host plants and Trolox standard at different concentration were plotted and tabulated and the IC50 for each of them was calculated using the BioDataFit fitting program in which the log sigmoidal fitting model was the adapted model.

RESULTS AND DISCUSSION

Antioxidant activity

There are a number of clinical studies suggesting that the antioxidant compounds in fruits and vegetables are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller and Rigelhof et.al (Miller *et al.,* 2000).

The free radical scavenging activity of the methanolic extract of the leaves of the wild and cultivated *Cyclamen persicum, Malva sylvestris* and *Urtica pilulifera* has been tested by DPPH radical method using Trolox as a reference standard. The concentration ranged from1–100µg/ml. The zero inhibition was considered for the solution which contained only DPPH without any plant extract. The results are shown in **Table (1)**, the table readings are presented in **Figure (4-A, B, C)**. The results show a difference in the anti oxidant activity for all samples. The more potent activity was for wild *Urtica pilulifera* extract (log IC50 10.2 µg/ml), and the cultivated *Urtica pilulifera* extract was comparatively lower with log IC50 of 29.9 µg/ml. Moreover, the antioxidant activity for the other plants were comparative with slight difference in the antioxidant activity; the wild plants of *Malva sylvestris* extract and *Cyclamen persicum* extract have slightly more antioxidant activity (log IC50 38.2 and 38.1 µg/ml) respectively; compared to the cultivated one (log IC50 40.2 and 39.8 µg/ml) respectively .

Table 1: Percentage inhibition activity for Trolox, Wild *Cyclamen persicum*, *Malva sylvestris*, *Urtica pilulifera* leaves and their cultivated Species

Conc: (µg/ml) DPPH/ Methanol	Inhibition by Wild Urtica extract (%)	Inhibition by Cultivated Urtica extract (%) inhibition	Inhibition by Wild Malva extract (%) inhibition	Inhibition by Cultivated Malva extract (%) inhibition	Inhibition by Wild Cyclamen extract (%) inhibition	Inhibition by Cultivated Cyclamen extract (%) inhibition	Inhibition by Trolox (%) inhibition
1	16.9	27.6	19.4	36.1	22.1	35.2	49.1
2	18.8	28.8	21.3	37.2	23.7	35.9	51.2
3	19.4	30.0	23.9	37.7	25.8	35.9	67.8
5	20.0	31.2	23.9	38.7	26.4	36.2	74.4
7	23.8	31.2	24.5	38.7	26.8	36.2	86.7
10	24.4	31.8	25.8	39.8	27.1	36.2	95.8
20	24.4	31.8	25.8	40.3	33.1	41.5	97.0
30	30.6	35.3	25.8	40.3	36.5	44.6	97.0
40	30.6	35.3	27.1	40.8	44.5	50.2	97.0
50	36.9	38.2	28.4	41.9	44.5	51.6	97.0
80	37.5	41.8	29.0	44.0	60.5	63.4	97.0
100	37.5	42.4	30.2	46.1	60.5	63.4	97.0
IC50	**10.2**	**29.9**	**37.1**	**40.2**	**38.2**	**39.8**	**5**

Table 2: Exhaustive aqueous extractions yields

Sample	Weight of sample	Weight of aqueous phase extraction yields	Percentage of aqueous phase extraction yields
Wild Cyclamen persicum	25g	8.84g	35.36
Cultivated Cyclamen persicum	25g	7	28
Wild Malva sylvestris	25g	10.5	42
Cultivated *Malva sylvestris*	25g	7.7	30.8
Wild Urtica pilulifera	25g	9.6	38.4
Cultivated *Urtica pilulifera*	25g	7.5	30

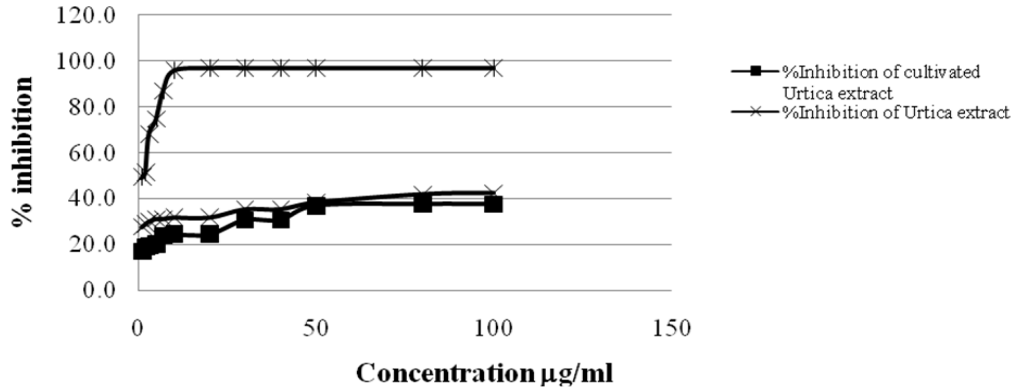

Fig. 4 (A): Inhibition activity of Trolox standard and Urtica extracts (cultivated and wild).

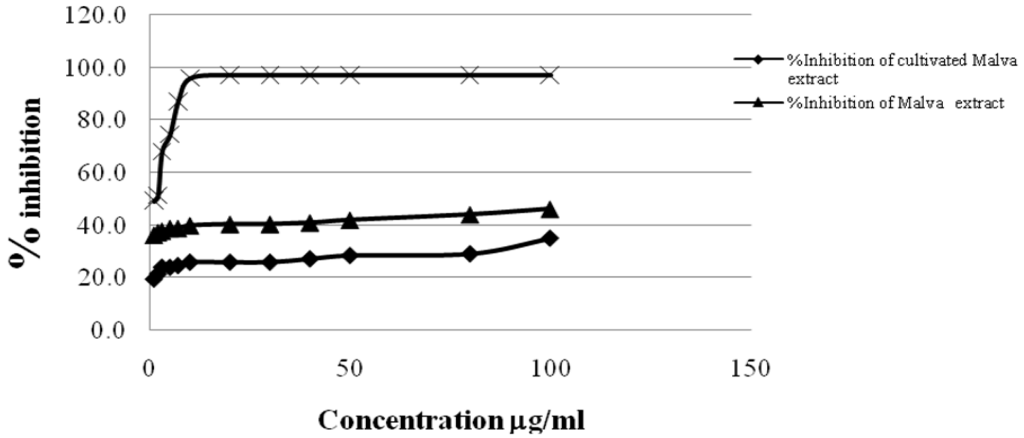

Fig. 4 (B): Inhibition activity of Trolox standard and Malva extracts (cultivated and wild)

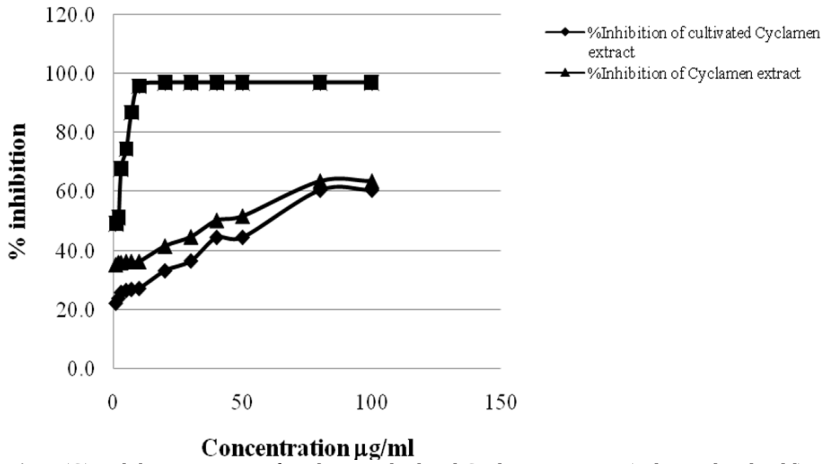

Fig. 4 (C): Inhibition activity of Trolox standard and Cyclamen extracts (cultivated and wild)

Table 3: Exhaustive organic extractions yields.

Sample	Weight of sample	Weight of organic phase extraction yields	Percentage of organic phase extraction yields
Wild Cyclamen persicum	25g	0.34g	1.36
Cultivated Cyclamen persicum	25g	0.25	1
Wild Malva sylvestris	25g	0.41	1.64
Cultivated Malva sylvestris	25g	0.29	1.16
Wild Urtica pilulifera	25g	0.61	2.44
Cultivated Urtica pilulifera	25g	0.42	1.68

The results clearly demonstrates the high antioxidant activity of *Urtica pilulifera* extracts specially the wild extract which was showed almost half antioxidant activity in comparison to Trolox standard (5.0 µg/ml).

The antioxidant activity of wild extracts for all the plants were close to their cultivated ones; this was against the expected results, it's thought that many factors like the watering and fertilizing of the cultivated plants could affect the antioxidant activity and the exhaustive extraction yield of the cultivated plants. For evaluation of exhaustive extractions yields the results showed that the natural wild growing *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* leaves have the highest aqueous and organic extracts yields as shown in tables (2 and 3).

CONCLUSION

The results clearly shows that the plants which have the highest organic and aqueous extraction yields have the highest antioxidant activities. Wild natural *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* plants leaves had slightly higher antioxidant activity than their cultivated species.

Based on the above presented results the rareness of natural antioxidant use which is usually due to the cost and unavailability of the wild plant could be overcome by using cultivated species. The cultivated *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* plants leaves, could be used as a possible new source of natural antioxidants in the food, nutraceuticals, pharmaceuticals and cosmetic industry.

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