Antioxidant evaluation for *Urtica urens, Rumex cyprius* and *Borago officinalis* edible wild plants in Palestine

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Abstract: Natural plants products are one of the famous and commonly utilized remedies used in fighting diseases. This study was conducted to evaluate antioxidant activity of plants commonly used in Palestine (*Urtica urens, Rumex cyprius* and *Borago officinalis*). Free radical scavenging activity method was evaluated by using 2,2-diphenyl-1-picryl-hydrazyl-hydrate. The result show that the overall antioxidant activity of *Rumex cyprius* was the highest among the plants, followed by *Urtica urens,* and *Borago officinalis*; respectively. The (IC_{50}) values of the methanolic extracts were 29.70±0.60µg/ml, 5.07±0.49µg/ml, 39.92±0.52µg/ml for *Urtica urens*, *Rumex cyprius* and *Borago officinalis* respectively. The results of this study revealed that these edible plants have high antioxidant activity and therefore they can provide natural sources of antioxidants and can be useful in preventing various diseases including cancer. These exhibited properties propose that such plants extracts can possibly be used as natural preservatives in the food and pharmaceutical industries and further characterization of *Rumex cyprius* constituents is needed.

Keywords: Wild edible plants, *Rumex cyprius, Urtica urens, Borago officinalis*, DPPH, antioxidant.

INTRODUCTION

Herbal extracts have been used in traditional medicine thousand years ago, nowadays there is an increasing interest in screening of the natural plant products and their folk uses (Verma and Singh, 2008). Moreover, the plant products are safe compared to synthetic substances (Kumar *et al.*, 2013). According to the World Health Organization (WHO); 80% of the patients depend on traditional herbal medicines for their health care needs (WHO, 2002). Natural antioxidant products have many biomedical application; this led to rising in screening for bioactive compounds with powerful antioxidant activity (Rishton, 2008).

An antioxidant compounds have the ability to trap free radicals and oxygen species that are present in biological systems (Viswanad *et al.*, 2011). Thus antioxidant in food products play an important role as a health protecting the body the from oxidative damage which may eventually lead to many chronic diseases such as, cancer, diabetics, rheumatoid arthritis, cardiovascular diseases, chronic inflammation, aging and other degenerative diseases (Fang *et al.*, 2002; Asaduzzaman Khan *et al.*, 2010; Schönthal, 2012) . Traditionally, wild edible plants used in the Mediterranean diet were cooked before consumption but still retain their antioxidant activity (Boari *et al.*, 2013).

Starflower "*Borago officinalis* L." belonged to the *Boraginaceae* family. It is an annual herbaceous plant that occurs during November to January (fig.1). It is originated in Syria and also available throughout Mediterranean region, Asia Minor, Europe, North Africa and South America (Galle *et al.*, 1993; ThePlantList, 2013). Starflower is cultivated for its culinary and medicinal uses. It is commercially cultivated for its seeds oil which contains gamma-linolenic acid and other fatty acids. Some naturopathic practitioners used it to regulate metabolism and the hormonal system and used it to treat menopausal symptoms such as the hot flash (Gupta and Singh, 2010). Sometimes it was indicated for treatments of colds, bronchitis and respiratory infections as well as it had anti-inflammatory effect (Farhadi *et al.*, 2012). However, the knowledge of anti-oxidative/ antiradical properties of crude extract of Starflower leaves is little compared to other plant activities. Starflower was investigated mostly for their medicinal properties for its oil due to its high content of γ-linolenic acid (Huang *et al.*, 1995; Bandonien and Murkovic, 2002). The antioxidant properties of the Palestinian borage extract has never been investigated by any method including the DPPH method (Wettasinghe and Shahidi, 1999).

Nettle (Stinging nettle), *Urtica urens* L. belonged to *Urticaceae* family and naturally growing in the pathways, fields and wildwood in Palestine. They are grown in mild climate areas, bottom of barriers, between cultivated plants, street, and water runnels. It has a wide distribution in the world (Otles and Yalcin, 2012). Nettle leaves are stinging, dark green and serrated edged. The leaves are 2-4 cm long, oval and core in shape (fig. 2). The flowers of nettle are small and green. The fruits of nettle are arid with single germ.

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Fig. 1: *Borago officinalis* plant

Fig. 2: *Urtica urens* L. plant

Fresh nettle can cause burning and blushing of skin if touched (Upton, 2013). Traditionally Nettle is used in the treatments of muscle pains, eczema, arthritis, gout, hypertension, anemia as well as used in the treatment of symptomatic benign prostate hyperplasia (Kirchhoff, 1983; Dreikorn, 2002; Safarinejad, 2005; Bent *et al.*, 2006).

Pink Sorrel (Dockor or Murb), *Rumex cyprius* belonged to the *Polygonaceae* family, which is an annual herbaceous plant growing wildly in the pathways, fields, and wildwood in Palestine (fig. 3). It is widely used in the Palestinian ethno medicine to treat skin diseases including dermatophytoses. Its antifungal effect was tested against four pathogenic fungi namely: *Microsporum canis, Trichophyton mentagrophytes* and *T. rubrum* and the result showed that it had a considerable activity against all these fungi (Ali Shtayeh and Abu Ghdeib, 1999; Abutbul *et al.*, 2005; Yıldız *et al.*, 2008; Husein *et al.*, 2012). Moreover, the antibacterial and antiviral activities of *R. cyprius* were assessed; it showed it had anti antibacterial as well as anti viral activity (Vermani and Garg, 2002; Abutbul *et al.*, 2005). All these studies have been done on *U. dioica* species but not one of them has been done on *U. urens* and antioxidant activity was not measured in the previous experiments by DPPH method for the other two plants.

Fig. 3: *Rumex cyprius* plant

To evaluate the antioxidant capacities of plant extracts, several in vitro methods are available. These methods include Oxygen Radical Absorbance Capacity (ORAC) method, Total Radical Trapping Antioxidant Parameter (TRAP) method, Trolox Equivalent Antioxidant Capacity (TEAC) method, Total Oxyradical Scavenging Capacity (TOSC) method, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, Peroxyl Radical Scavenging Capacity (PSC) method, and others (Senevirathne *et al.*, 2006; Mermelstein, 2008). The DPPH method is a UV method in which the free radical has a purple color and absorbs strongly at 517nm. When the DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant it will be reduced to DPPH-H, the color becomes yellow and the absorption of the DPPH at 517 nm reduces (Prakash *et al.*, 2007). The antioxidants activity is usually compared with a reference standard such as Trolox. It is which is a trade name for 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid, it is a water soluble vitamin E analogue used in this research as an antioxidant standard (Prior *et al.*, 1998).

The objectives of this research are to evaluate the antioxidant activity for *U. urens, R. cyprius* and *B. officinalis* and to compare between the antioxidant activities of these three plants.

MATERIALS AND METHODS

Reagents

Methanol analytical grade was used for extraction purposes. Trolox ((S)-(-)-6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) were purchased from Sigma Aldrich Chemical Co., Gillingham, Dorset, UK. All the other chemical reagents that we used were of analytical grade and were purchased from reliable commercial sources.

Instrumentation

Shaker device (Lab Tech Shaking Incubator) was used in extraction of the plants, rotatory evaporator (Heidolph OB2000). The heater and the rotator (Heidolph VV2000) were used for condensation purpose. The spectrophotometer (Jenway 6505 UV/Vis Spectrophotometer) was used to measure the optical density.

Plant material

Leaves of *U. urens*, *R. cyprius* and *B. officinalis* were collected from the West Bank area of Palestine. The plants were botanically identified by pharmacognosist Dr. Nidal Jaradat. Voucher specimens were deposited in the laboratory of Pharmacognosy at the Pharmacy Department, An-Najah National University and their voucher specimen code was (Pharm-PCT-2562) for *U. urens*, (Pharm-PCT-2070) for *R. cyprius* as well as it was (Pharm-PCT-2745) for *B. officinalis*. These studied plants were then dried in dark places. The dry plants were then stored in dry bottles for further research that was performed in summer 2013.

Extract preparation

Leaves of the plants (*U. urens*, *R. cyprius* and *B. officinalis)* were powdered separately using a mechanical grinder. The extraction was performed at room temperature. About 100g of the grounded 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 proper filter papers. The filtrate was then concentrated under vacuum on a rotary evaporator. The crude extract was stored at 4ºC for further use.

Standard and plant working solutions

A stock solution of a concentration of 1mg/1ml in methanol 99% was firstly prepared for the two 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.

Test measurements

The DPPH was freshly prepared at a concentration of 0.002% w/v, 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 room for 30 minute at room temperature before the absorbance readings were recorded at 517nm.

Trolox ((S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) were purchased from Sigma Aldrich. Methanol analytical grade was used for extraction purposes. Other chemical reagents were methanol (99%) purchased from reliable commercial sources.

Plant antioxidant activity

The percentage of antioxidant activity of the plants and the Trolox standard were calculated using the following formula:

$$\text{Percentage of inhibition of DPPH activity (\%)} = \frac{A - B}{A} \times 100\%$$

Where: A = optical density of the blank,
B = optical density of the sample.

The antioxidant half maximal inhibitory concentration (IC_{50}) for the plant samples and the standard were calculated using Bio Data Fit (BioDataFit, 2002). The extract that has the highest IC_{50} parameter for the scavenging activity of the free radical show highest antioxidant activity.

RESULTS

The ground leaves from each host i.e. the plants were weighed 100 g each, the methanolic extraction of Nettle, Pink Sorrel and Starflower yielded 1.88g, 2.94g and 2.65g respectively. The extracts were thick viscous residues, which have a dark greenish color, and were kept in its dry viscous state at room temperature for further experimental work.

In this research, DPPH method, which relies on the reduction of 2,2- diphenylpicrylhydrazyl (DPPH) radical was used. This method is simple, fast and inexpensive for measuring the antioxidant capacity. Furthermore, it is not specific to any particular antioxidant component and could be applied to either solid or liquid samples. The free radical scavenging activity of the methanolic extract of the plants has been tested by DPPH radical method and using Trolox as a reference standard. The concentration ranged from 1-100µg/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 results show that the anti-oxidant activity reaches a platue at a concentration of 100µg/ml for Trolox standard, but for the tested plant none has reached it at that concentration. The results show also the difference in antioxidant activity for the three host plants. In order to make a comparison study between the three plants and the Trolox extract, it was necessary to calculate the inhibition concentration (IC_{50}), which is the concentration of the compound at which it reaches half of its maximum inhibitory effect. When the value of the IC_{50} is less, this means that it has a greater effectiveness as an anti-oxidant. Using Bio Data Fit program, the IC_{50} was calculated table 2. The results show that the antioxidant

Table 1: The calculated percentage of inhibition activity of Trolox standard and the three plants; Stinging nettle, Sorrel and Starflower.

Concentration (µg/ml)	(% inhibition) Trolox	(% inhibition) *U. urens*	(% inhibition) *R. cyprius*	(% inhibition) *B. officinalis*
1	49.09	34.04	41.6	35.6
2	51.20	34.04	47.4	36.7
3	67.77	34.45	52.1	38.2
5	73.39	36.03	56.2	38.6
7	86.75	37.22	60.4	40.5
10	95.78	37.6	62.5	40.7
20	96.68	38.4	64.58	40.7
30	96.68	41.9	66.6	41.6
40	96.68	42.39	68.6	47
50	96.68	43.58	68.7	47
80	96.68	45.17	70.8	56.6
100	96.68	48.35	70.8	57.4

Table 2: The antioxidant activity of methanolic extract of Trolox, *U. urens*, *R. cyprius* and *B. officinalis*.

Methanolic extract	Log IC_{50} (µg/ml) (Mean ± SD)
Trolox	5.10±0.32
U. urens	29.67±0.60
R. cyprius	5.07±0.49
B. Officinalis	39.92±0.512

activities for the three plants were comparatively lower compared to the Trolox reference standard, which is known of its huge antioxidant activity.

Among the extracts of the three plants; *U. urens, R. cyprius* and *B. officinalis* standard tested for the antioxidant activity using the DPPH method, the methanolic extracts of Trolox had shown the highest antioxidant activity. The results indicate that, *R. cyprius* was the most active radical scavenging plant resulting in, followed by *U. urens* and lastly *B. officinalis*.

DISCUSSION

Herbal drugs represent more than 50% of all the drugs in modern therapeutics. In addition to their use in alternative medicine, wild plants are eaten freshly or after cooking by different communities around the globe. Moreover, the antioxidant capacity of different plants, in addition to the plants' phenols and flavonoids content, gives the plant their significant nutritional and therapeutic values (Pan *et al*., 2013).

In comparing with an experimental study on another species of Nettle leaves (*U. dioica*) which is the most commonly used species of Stinging nettle world widely and one of the oldest medicinal plants, that was performed by Chahardehi *et al*, 2009, on its leaves, the results showed that its antioxidant activity was 62.537µg/ml while our studied plant its antioxidant activity 29.67 µg/ml, which indicated that our studied Urtica plant has more potential antioxidant activity than *U. dioica* plant (Chahardehi *et al.*, 2009).

Recently and to the best of our knowledge there are no previous antioxidant studies on *R. cyprius* leaves and our study is the first one. In another study evaluated antioxidant activity for *Borago officinalis* leaves performed by Conforti *et al*., 2008, the results showed that Borage plant growing in Palestine had more powerful antioxidant activity than the studied Italian Borage (Conforti *et al.*, 2008).

CONCLUSION

The results of this study revealed a significant difference in the antioxidant activity between the three plants. *R. cyprius* was the most active antioxidant plant. This suggests a careful consideration of these plants especially the sorrel when administering this medical and edible plant. Further research must be done using sophisticated separation technique to know the exact constituent of this plant. The isolated components then can be identified and tested separately and then can be used for manufacturing new antioxidant safe pharmaceutical forms.

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