Current Topics in Biophysics 2000, 24(2), 219–224

ESTIMATION OF TOTAL ANTIOXIDANT POWER IN MEDICINAL PLANTS (ADAPTATION OF FRAP METHOD) ANTIOXIDANT POWER IN MEDICINAL PLANTS

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The intake of antioxidants present in food is an important health-protecting factor. Herbal compounds known by ancient medicine are of growing interest in the domain of prevention of diseases. Medicinal plants have a lot of antioxidants. mostly polyphenols, flavonoids that exhibit high antioxidant activity. Total antioxidant activity values were determined from fresh plant samples by the FRAP method. FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593 nm. The aim of our work was to get answer for the question: is this method applicable for investigation of fresh plant samples and herbs? Several type of medicinal plants were involved in our investigations: from *Labiatae* family *Melissa officinalis* L., *Ocimum basilicum* L., and *Satureja hortensis* L., from *Urticaceae* family *Urtica dioica* L. and from *Papaveraceae* family *Chelidonium majus* L. Our results show that FRAP method is sensitive in the measurement of total antioxidant power of fresh biological fluids, such as plant homogenates and pharmacological plant products.

INTRODUCTION

Living organisms have developed a complex antioxidant network to counteract reactive oxygen species that are detrimental to human life. When free radical production exceeds antioxidant defenses, these radicals react with all types of biomolecules, including lipids, proteins and nucleic acids (Halliwell & Gutteridge, 1985). The oxidation products of these macromolecules can directly or indirectly produce tissue injuries. Oxidative stress can be reduced with the provision of additional antioxidants. According to numerous studies, such antioxidants are closely related with the prevention of degenerative illness, such as cardiovascular, neurological diseases, cancer, and oxidative stress dysfunction (Halliwell, 1996; Bolck, 1992; Diplock, 1995).

Foods of plant origin not only provide us with important antioxidant vitamins, such as vitamin C (ascorbic acid), vitamin E (α-tocopherol) and provitamin A (β-carotene), but also a complex mixture of other natural substances with antioxidant capacity. It is not surprising therefore, that plant extracts were successfully used in phytotherapy since ancient times.

It is possible to measure all of the antioxidant components in a sample individually, but this is both time-consuming and expensive. The possible interaction among different antioxidants *in vivo* could also make the measurements of any individual antioxidant less representative of the overall antioxidant status.

Methods developed for total antioxidant capacity of biological samples have been classified as inhibition methods involving reactive species (Wayner, Burton, Ingold & Locke, 1985; Millner, Rice-Evans, Davies, Gopinathan & Milner, 1993; Cao & Prior, 1999). FRAP assay, which was elaborated for human plasma, depends upon the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593 nm (Benzie & Strain, 1996). The aim of our work was to get answer for the question: is this method applicable for investigation of fresh plant samples and herbs?

Several type of medicinal plants were involved in our investigations: from *Labiatae* family *Melissa officinalis* L., *Ocimum basilicum* L., and *Satureja hortensis* L., from *Urticaceae* family *Urtica*

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Table 1. What we know of plants today

Informations	*Melissa officinalis*	*Ocimum basilicum*	*Satureja hortensis*	*Urtica dioica*	*Chelidonium maius*
Folk medicine uses as/in	Component of tea mixture, appetizer, sedative, rheumatism	Appetizer, purgative agent spice	Expectorant, appetizer, diarrhea, spice	Asthma, allergy, Bronchitis External use: Falling hair, Dandruff	Against wart and corn, sedative, cholagogue, analgesic, spasmolytic
New statement	Antiviral effect (Herpes simplex 1) anti-HIV-1 activity	Inhibits the HIV-1 reverse transcriptase	Bactericid, fungicid effect	Lung-, liver-, stomach-diseases	Inhibits mitosis and tumor growing
Main antioxidant compounds	Rosmarinic acid, catechin Volatile oils	Mostly volatile oils – 0.4%	Tannin, volatile oils, E.g.carvacrol	Flavonoids, terpenoids, histamine, formic acid...	Chelidonine, berberine, sparteine
Drug	Melissa herba (blooming shoot)	Basilici herba	Saturcja herba	Urticae folium, – radix, – fructus, – herba	Chelidonii herba, – radix
Collection time	July-September	June-September	June-September	May-September	June-September

dioica L. and from *Papaveraceae* family *Chelidonium majus* L.

Plants

What we know of plants today is summarized in Table 1.

METHODS

All chemicals and reagents were analytical grade or purest quality purchased from Sigma, Merck, Aldrich, Fluka or Reanal (Budapest, Hungary).

Plants were collected in the Botanic Garden of the University of Szeged or along the roads in the beginning of the vegetation period and regularly to the end of the vegetation period. Botanic Garden was considered as place without any environmental pollution.

1 g of leaves, shoot or stem were cut into small pieces and mashed with a cool mortar and pestle using quartz sand and 9 ml cool 0.1 M phosphate buffer (pH 7.6, containing 0.1 mM EDTA). This mixture was filtered through a filter paper and centrifuged at 15000 rpm for 10 min. The supernatant was used for the measurements.

The method for measuring the ferric reducing ability of plasma (FRAP) or with other words the measurement of "antioxidant power" was published by Benzie and Strain (1996). We modified this automated method for manual assay (Varga, Matkovics, Sasvári & Salgó, 1998).

Our protocol is shortly as follows:

Reagents:

1. Acetate buffer, 300 mmol/l pH 3.6 (3.1 g sodium acetate × $3H_2O$ and 16 ml conc. acetic acid per l of buffer solution).

2. 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl

3. 20 mmol/l $FeCl_3$ × $6H_2O$ in distilled water (d.w.).

FRAP working solution: 25 ml acetate buffer (1), 2.5 ml TPTZ solution and 2.5 ml $FeCl_3$ × $6H_2O$ solution. The working solution must be always freshly prepared.

Aqueous solution of known Fe (II) concentration was used for calibration (in a range of 100–1000 µmol/l).

Assay: Blank: FRAP reagent.

Sample: FRAP reagent 1.5 ml,
Plant extract 50 µl.

Monitoring up to 5 min at 593 nm, 1 cm lights path and 25°C. Calculation: using the calibration curve. The relative activities of samples were assessed by comparing their activities with that of Trolox® (Hoffman-LaRoche) or L-ascorbic acid.

RESULTS

Results of our investigations are summarized in Figures.

Total antioxidant activities of investigated plants show maximum values two times during the vege-

tation period, at the blooming time and in the storage period.

Changes of the antioxidant activity (FRAP value) of parts of *Melissa officinalis* L. were balanced except in July, when we measured high activities in the shoot and the stem and low activity in the leaves (Fig. 1A) The tendencies of changing almost the same regarding the Trolox equivalent antioxidant activities (Fig. 1C). The ascorbic acid

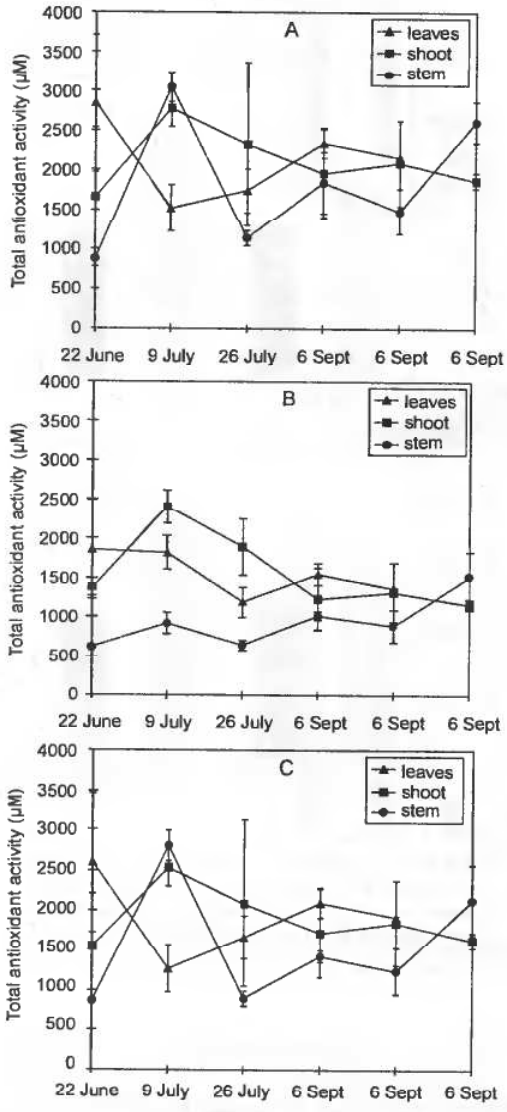

Fig. 1. Ferric reducing ability (FRAP) of several parts of *Melissa officinalis* L. (A), ascorbic acid equivalent antioxidant activities (B) and Trolox equivalent activities (C) of the same samples. Plants collected in June were before blooming, in July when blooming and during late blooming, and in September we tested an old plant with second blooming, new shoot of the old plant and old plant after blooming, respectively

(AA) equivalent antioxidant activities were the highest in the floral shoot, approximately equal to the sum of the activities of the other two parts. These high values were due to the accumulation of secondary metabolic products e.g. rosmarinic acid. French researchers (Lamaison, Petitjean-Freytet & Carnat, 1991) proved free radical scavenging activity of the rosmarinic acid. Others demonstrated the presence of caffeic, rosmarinic and ferulic acids in *Melissa officinalis* L. and the role of these compounds in the antiviral activity of plant was proven (Dimitrova, Dimov, Manolova, Pancheva, Ilieva & Shishkov, 1993). The rise in the antioxidant activities in the leaves and shoots after blooming period depend on the appearance of fresh shoots or coming storage processes. Looking at our results, the best term for collection is July.

Total antioxidant activities in *Ocimum basilicum* L. show similar tendencies (Fig. 2). The FRAP values and Trolox equivalent activities were high in the leaves and stem before blooming. (Fig. 2A and C). To use as culinary plant, this is the best period because of its high volatile oil content (Deans, Noble, Pénzes & Beregi, 1989). Shoot samples of basil, which used as drug in the medicine, showed the highest activities in July, but AA equivalent activities were highest in June before blooming. Plant extract of *Labiatae* species, e.g. *Ocimum basilicum* L. and *Melissa officinalis* L., showed significant inhibitory effect against HIV-1 induced cytopathogenicity in MT-4 cells (Yamasaki, Nakano, Kawahata, Mori, Otake, Ueba, Ioshi, Inami, Yamane, Nakamura, Murata & Nakanishi, 1998).

The folk medicine gives term to collect *Satureja hortensis* L. herbs from June to September. We measured the total antioxidant activities in June and in July. Both of the investigated plants were flowering (Fig. 3A, B and C). The FRAP values and the relative antioxidant activities were highest in the shoot in June and in the leaves in July. It is reasonable to collect leaves used up in the kitchen in July.

The total antioxidant activities of the next two plants, *Urtica dioica* L. (Fig. 4) and *Chelidonium majus* L. (Fig. 5), were followed up from spring to late autumn. The FRAP values were decreased at the beginning of the reproductive period and started to increase in June. We got severe differences in antioxidant activities of plants collected from different places. The metabolic pathways are influenced by the quality of the soil, environmental pollution, meteorological circumstances etc. So, we can say that by testing the total antioxidant

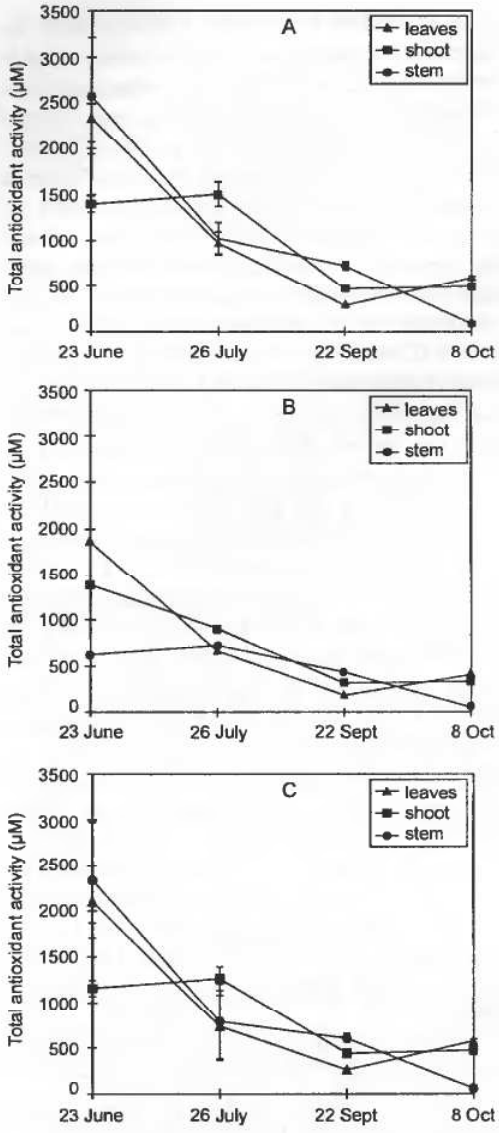

Fig. 2. Total antioxidant activity values of several parts of *Ocimum basilicum* L. FRAP values (A) are expressed as acid equivalent antioxidant activities (B) and Trolox equivalent activities (C). Plant samples collected at the end of June were before blooming, in July were blooming and in September and October we tested old plants

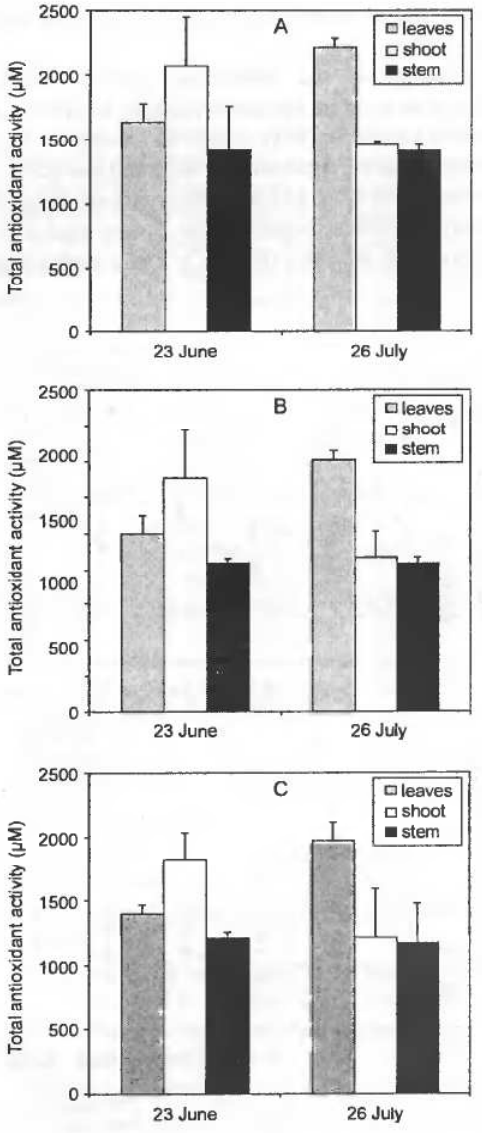

Fig. 3. Ferric reducing ability (FRAP) of leaves, shoot and stem of *Satureja hortensis* L. (A), ascorbic acid equivalent antioxidant activities (B) and Trolox equivalent activities (C) of the same samples. Plants investigated in both times were blooming

activity we can get information about the metabolic changes.

Both plants enjoy great interest in phytomedicine. Commune stinging nettle was used for treatment of joint pain (Randall, Meethan, Randall & Dobbs, 1999). *Chelidonium* extracts have antispasmodic and relaxant activity (Hiller, Ghorbani & Schilcher, 1998) and preventive effects with respect to gland tumor development (Kim, Ahn, Han & Tsuda, 1997).

DISCUSSION

Several papers have been published recently comparing the different methods for total antioxidant activity measurement (Varga *et al.*, 1998; Prior & Cao, 1999). The principles of the methods are oxidation-reduction changes i.e. one electron transfer. Most of the methods have been classified as inhibition methods; they involve a pro-oxidant (which is usually a free radical) and an oxidizable

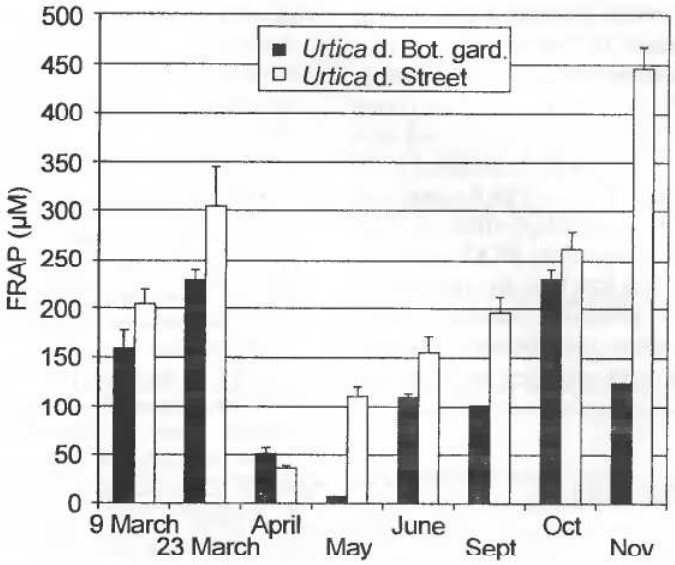

Fig. 4. Changes in total antioxidant activity of *Urtica dioica* L. leaves during the vegetation period. We collected plants in the Botanic garden (as a clean place) and from the street (as a polluted place)

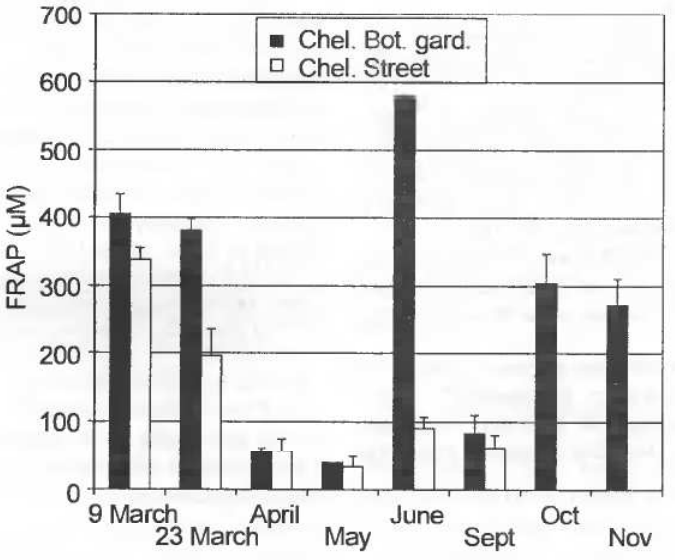

Fig. 5. Total antioxidant activity values in *Chelidonium majus* L. leaves measured from March to November. To compare the effects of environmental pollution we collected plants from the Botanic garden and from the street

substrate. The pro-oxidant induces oxidative damage to the substrate, which is inhibited in the presence an antioxidant. Some of these methods use of peroxyl or hydroxyl radicals as prooxidants e.g. in the oxygen radical absorbance capacity (ORAC) assay (Cao & Prior, 1999), or TRAP (total radical trapping parameter) assay (Wayner *et al.*, 1985). Both methods use peroxyl radical generated from 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH).

Miller *et al.* (1993) reported the TEAC (Trolox equivalent antioxidant capacity) assay. This assay based on the inhibition by antioxidants of the absorbance of the ABTS cation [2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate)].

The FRAP assay involves neither a pro-oxidant nor an oxidizable substrate. This method depends upon the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant (Benzie *et*

al., 1996). What we really measure is the ability of a compound to reduce Fe^{3+} to Fe^{2+}. The ferric-reducing ability measured for a biological sample may indirectly reflect the total antioxidant power of the sample. The FRAP assay is quick and simple to perform and reagents are inexpensive.

Our results support that the FRAP method is suitable to assess the antioxidant effect of fresh plant samples. Making use of the FRAP method it is possible to choose the best time for collection of plants. There is the possibility therefore, that a number of these plants may possess sufficient antioxidant capacity to be exploited for promotion of human health.

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