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Total antioxidant power in some species of Labiatae (Adaptation of FRAP method)

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ABSTRACT Medicinal plants have a lot of type antioxidants, mostly polyphenols, flavonoids which exhibit high antioxidant activity (Rice-Evans et al. 1995). 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. The FRAP assay (ferric reducing ability of plasma), a simple test of the total antioxidant power have been chosen to assess the presumable effects of some kind of tea and medicinal plant. The aim of our work was to get answer for the question: is this method applicable for investigation of fresh plant samples and herbs? FRAP assay depends upon the 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 colour and can be monitored at 593 nm. (Benzie and Strain 1996). Several species of medicinal plants were involved in our investigations: from *Labiatae* family *Melissa officinalis*, *Mentha piperita, Ocimum basilicum*, *Salvia officinalis, Satureja hortensis and Majoranna hortensis.* 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. Antioxidant activity of our samples were confirmed with *in vitro* model system.
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KEY WORDS

antioxidant activity
FRAP assay
polyphenols
flavonoids

Oxidative stress can be reduced with the provision of additional antioxidants. Antioxidants are closely related with the prevention of degenerative illness, such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunctions (Bolck 1992; Diplock 1995; Halliwell 1996).

Foods of plant origin not only provide us with important antioxidant vitamins (*e.g.* vitanim C, vitamin E or provitamin A), but also a complex mixture of other natural substances with antioxidant capacity. It is possible to measure all of the antioxidant components in a sample individually, but this is expensive and time-consuming.

Several methods are known to measure the total antioxidant capacity of biological samples, but we tried the FRAP assay, which 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 colour and can be monitored at 593 nm (Benzie and Strain 1996). Although this method was elaborated for human plasma, we wanted to get answer to the question: is this assay applicable for investigation of fresh plant samples and herbs?

We involved in our investigations some well-known medicinal plants from *Labiatae* family: *Melissa officinalis L.*, *Mentha piperita L., Ocimum basilicum L.*, *Salvia officinalis L., Satureja hortensis L. and Majoranna hortensis Mönch.*

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

Materials and Methods

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

One 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 15.000. rpm for 10 min. The supernatant was used for the measurements.

The automated method for measuring the FRAP or with other words the measurement of "antioxidant power" was modified by Varga et al. (1998) to a manual assay.
Our protocol is shortly as follows:
Reagents:
1) Acetate buffer, 300 mmol/l pH 3.6 (3.1g sodium acetate x 3 H_2O and 16 ml conc. acetic acid per 1 of buffer solution).
2) 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl.
3) 20 mmol/l $FeCl_3$ x 6 H_2O in distilled water (d.w.).
FRAP working solution: 25 ml acetate buffer (1), 2.5 ml TPTZ solution and 2.5 ml $FeCl_3$ x 6 H_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 ml.

Monitoring up to 5 min at 593 nm, 1 cm lights path and 25ºC. 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.

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Table 1.

Informations	*Melissa officinalis*	*Mentha piperita*	*Ocimum basilicum*	*Satureja hortensis*	*Salvia* officinalis	*Majoranna* hortensis
Folk medicine uses as/in	Component of tea mixture, appetizer, sedative, rheumatism	Appetizer, analgetic, bronchitis	Appetizer, purgative agent, spice	Expectorant, appetizer, diarrhea, spice	Parodontosis, spice	Spice, gastritis
New statement	Antiviral effect (Herpes simplex 1), anti-HIV-1 activity	Antiseptic	Inhibits HIV-1 reverse tanscriptase	Bactericid, fungicid effect	Antibiotic	Antiphlogistic, antirheumatic
Main antioxidant compounds	Rosmarinic acid, catechin, volatile oils	Rosmarinic acid, coffeic acid, volatile oils	Mostly volatile oils- 0,4%	Tannin, volatile oils (*e.g.* carvacrol)	Rosmarinic acid, volatile oils, catechin, coffeic acid	Volatile oils, rosmarinic acid
Drug	Melissae herba (blooming shoot)	Menthae piperitae folium	Basilici herba	Saturejae herba	Salviae folium	Majoranae herba
Collection time	July-September	July-September	June-September	June-September	July-September	July, September-October

Results

Total antioxidant activities of investigated plants show maximum generally two times during the vegetation 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 (Table 2).

The tendencies of changing almost the same regarding the Trolox equivalent antioxidant activities. The ascorbic acid (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 reseachers (Lamaison et al. 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 et al. 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.

Data of measurements of *Mentha piperita* L. showed the similar changes: the FRAP values were low in the leaves, shoot and stem before blooming, then were higher during the blooming period, finally got lower again after blooming. The antioxidant activity of leaves were the highest in the blooming time perhaps because of the great amount of rosmarinic acid and its fenolic OH-groups with reducing effect. After all we think that the ideal collection time is September.

Total antioxidant activities on *Ocimum basilicum* L. show similar tendencies to *Melissa* off. (Table 3).

The FRAP values and Trolox equivalent activities were high in the leaves and stem before blooming. To use as culinary plant, this is the best period because of its high volatile oil content (Deans et al.1989). Shoot samples of basil, which is 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 cytopathogenecity in MT-4 cells (Yamasaki et al. 1998).

Our samples of *Salvia officinalis* L. were taken from 1-year-old plant. Although the flowering time of older ones is in June and July, this way, without blooming the FRAP values of the stem and shoot were high through blooming, at the end of September it decreased only in the shoot (the

Table 2. FRAP values.

Melissa off.	22 June	9 July	26 July	6 Sept
leaves	high	↓↓	↑	↑
shoot	middle	↑	↓	↓
stem	low	↑↑	↓↓	↑

Table 3. FRAP values.

Ocimum basilicum	23 June	26 July	22 Sept	8 Oct
leaves	middle	↓↓	↓	↑
shoot	low	↑	↓↓	↑
stem	high	↓↓	↓	↓

equivalent graphs showed the similar tendencies). September is the second collection time and it is reasonable because the amount of the antioxidants (picrosalvin, rosmarinic acid) is getting higher this time (Deans et al. 1993).

The folk medicine gives term to collect *Satureja hortensis* L. herbs from June to September. We measured the total antioxidant activities in June and July. Both of the investigated plants were flowering. The FRAP values and the relative antioxidant activities were the 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 last investigated plant was *Majoranna hortensis* Mönch. We measured high antioxidant activities in the leaves and stem during the first part of the blooming period (July), but these values got lower by the second collection time (September-October) even if we expected them higher. The high values were because of the presence of rosmarinic acid (Lamaison et al. 1991).

Several papers have been published recently comparing the different methods for total antioxidant activity measurement (Varga et al. 1998; Prior and Cao 1999). We used FRAP assay which was elaborated by Benzie and Strain for human cases originally (Benzie and Strain 1996). Nevertheless this method is appropriate to measure the total antioxidant capacity and state of medicinal herbs if we want to use them in phytotherapy.

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