

## Antioxidant properties of tablets prepared from ginkgo, echinacea and mentha dry extracts

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### Abstract

*In order to evaluate the antioxidant activity of ginkgo, echinacea and mentha dry extracts, as well of the tablets prepared from these three, the followings have been investigated: total phenolics and flavonoids content, scavenging capacity on DPPH radical and reducing power. The highest content of phenolic compounds was detected in mentha extract, 180.23 mg GAE/g. The content of total phenolic compounds in 1 g of tablet was 23.29 mg GAE. The total flavonoids/total phenols ratio (TF/TP) in tablest was around 35%, higher than in the case of mentha and ginkgo extracts. Radical scavenging activity was found to exhibit 50% of inhibition value (IC<sub>50</sub> value) at the extract concentration of 0.015±0.007 mg/ml for investigated mentha extract. IC<sub>50</sub> values for ginkgo and echinacea extracts were lower than 0.01 mg/ml. For prepared tablets this value was 0.029 mg/ml. They all possess reductive capabilities, and some of them are comparable to reductive capabilities of synthetic antioxidant Trolox.*

**Key words:** ginkgo, echinacea, mentha, tablets, antioxidants

**Abbreviations:** CE – catehin equivalent; DPPH-1,1-Diphenyl-2-picryl-hydrazyl-hydrate; GAE – gallic acid equivalent; IC<sub>50</sub> - 50% of inhibition value; RSC – radical scavenging capacity; TF – total flavonoids content; TP – total phenols content; UV-VIS - Ultraviolet-visible spectroscopy.

### Introduction

Disturbance of the balance between production of free radical and activity of antioxidant systems of protection causes the so called oxidative stress which further results in numerous diseases. Many edible plants are capable of producing natural antioxidant, phytochemical compounds that are able to remove damaging radical species, as shown by a range of *in vitro* assays. Humans utilize a wide range of antioxidants that are either synthesized *de novo* or obtained from the diet. Phenolic secondary metabolites play an important role in plant-derived food quality, as they affect quality characteristics such as appearance, flavor and health-promoting properties (1).

As natural extracts are complex mixtures of compounds, the antioxidant activity is not the property of a single compound. It is important to determine which groups of compounds are most significant in determining antioxidant potency. The protective effect of fruits, vegetables and medicinal herbs are now linked to the presence of antioxidants, vitamins and phenolic phytochemicals having antioxidant activity, which support the body's antioxidant defense system (2).

Due to the presence of the conjugated ring structures and the number and arrangement of the polar hydroxyl groups (3) many phenolic compounds have potential to function as

antioxidants by scavenging superoxide anion (4), singlet oxygen (5), and lipid peroxy radicals (6), and by stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species (7). Most active phenolic antioxidants, due to their chemical structure, are flavonoids.

Considering the growing interest for natural antioxidants sources, the scope of this research was to investigate the antioxidant properties and some antioxidant compounds of ginkgo, echinacea and mentha, and of tablets prepared on the basis of these three extracts.

*Echinacea purpurea* L., also known as “purple coneflower” is an important medicinal plant, being widely used as an herbal drug. In modern cultures, echinacea is used as immunostimulant, bacteriostatic, anti-inflammatory and for wound healing properties, that mediate its indications mainly for upper respiratory tract diseases and infections (8-10). Many bioactive substances, polar and non polar, were isolated and characterized from the root or top parts of this plant: alkamides (or alkylamides), polyphenolic caffeic acid derivatives, polysaccharides, alkaloids, essential oils and many other miscellaneous structures (11, 12).

Ginkgo is a valuable tree, regarded as a “living fossil”, which belongs to the gymnosperm family (13). Modern research has shown that *Ginkgo biloba* extract can reduce blood pressure, dilates peripheral blood vessels, increases capillary and venous blood flow to the head (14, 15), improves viscoelasticity of blood (16), reduces oxidative metabolism in brain neurons (17), and scavenge radicals (18). Ginkgo is currently one of the most popular medicinal plants, and its phytopharmaceuticals are accessible throughout the world. Pharmacological studies reveal that ginkgo mainly contains two groups of active ingredients: flavonoids and terpenoids. Approximately 35 flavonoids have been isolated from *Ginkgo biloba* L. (19)

*Mentha* could be found in almost all parts of the world. It is a popular traditional medicinal plant, widely used as a tea. It is mainly used for respiratory ailments but many other uses have also been recorded such as for coughs, colds, stomach cramps, asthma, flatulence, indigestion and headaches.

The important properties of all three mentioned medicinal plants can be used together in the form of dietetic supplement, e.g. tablets.

In order to evaluate the antioxidant activity of prepared tablets and of dry extracts, the followings have been investigated: total phenolics and flavonoids content, scavenging capacity on DPPH<sup>•</sup> and reducing power.

## Materials and methods

### Chemicals and Reagents

1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Folin-Ciocalteu reagent, Gallic acid was purchased from Sigma (Sigma, St. Luis, MO, USA). Potassium ferricyanide, FeSO<sub>4</sub>, was purchased from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical reagent grade.

### Preparation of dry extracts and tablets

Liquid extracts of mentha, ginkgo and echinacea were prepared using multi level counter-current extraction by 40% ethanol as an extraction solvent. From 300 g of grind drug (mentha, ginkgo or echinacea), through five levels of extraction process, 1500 g of liquid extract was prepared. Obtained liquid extracts were dried in spray drier (APV Anhidro As, Denmark). Process parameters of drying process were: input temperature 125°C, output temperature 70-80°C and extract flow of 2l/h. This way dry extracts were prepared.

Tablets were prepared using three dry extracts: echinacea, mentha and ginkgo. Formulation of tablets included: lactose, starch, Aerosil, in the amount of 44.0 g, 25.0 g and

2.0 g, respectively, dry extracts of ginkgo, echinacea and mentha in amounts of 10.0g, 10.0g and 5.0 g, respectively. The ingredients were homogenized for 30 minutes, after two kinds of starch (water soluble starch and corn starch) were added as a binder. Obtained mixture was dried at the room temperature for 24 hours. After granulation, magnesium stearate was added, followed by a homogenization of 30 minutes. In this way a prepared mixture for tablets was obtained.

#### **Determination of antioxidation compounds**

The content of total phenolic compounds in dry extracts and tablets was determined by Folin-Ciocalteu procedure (20), using gallic acid as a standard. Absorbance was measured at 750 nm. Content of total phenolic compounds has been expressed as mg of gallic acid equivalent (GAE) per g of dry extract or tablets.

The total flavonoids content has been determined by aluminium chloride colorimetric assay (21), using catehin as a standard. It has been expressed as mg of catehin equivalents (CE) per g of dry extracts or tablets.

#### **DPPH assay**

The free radical scavenging capacity (RSC (%)) of extracts and tablets were determined as described by Espin (22). This assay was used for a preliminary free radical-scavenging evaluation. It is a simple and fast method in comparison to many others. DPPH<sup>·</sup> is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule (20). Decrease in absorbance of DPPH<sup>·</sup> radical is caused by a reaction between antioxidant molecules and the radical, which results in the scavenging of radical by hydrogen donation, what is visually noticeable as a discoloration from purple to yellow. Briefly, the extracts or tablets were mixed with methanol (96%) and 90 µM 2,2-diphenyl-1-picryl-hydrazyl (DPPH) to give the final concentrations 0.01 and 0.02 mg/ml of dry extract. After 60 min at room temperature, the absorbance was measured at 517 nm and was expressed as radical scavenging capacity. Radical scavenging capacity (RSC (%)) was calculated by equation (1).

$$RSC = 100 - (A_{\text{sample}} \times 100) / A_{\text{blank}} \quad (1)$$

where:  $A_{\text{sample}}$  is absorbance of sample solution and  $A_{\text{blank}}$  is absorbance of blank sample.

This activity was also expressed as the inhibition concentration at 50% (IC<sub>50</sub>), the concentration of test solution required to obtain 50% of radical scavenging capacity.

#### **Determination of reducing power**

The reducing power of extracts and tablets, as well as of ascorbic acid and Trolox, standard antioxidants, was determined by Oyaizu method (23). Various concentrations of extracts and tablets (0.01, 0.025, 0.05, 0.1, 0.2, 0.5, and 1 mg/ml), Trolox and ascorbate (0.01, 0.025, 0.05, 0.1 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>]. The mixture was incubated for 20 minutes at 50°C. After incubation, 2.5 ml of 10% trichloroacetic acid solution was added to the mixture, mixture was centrifuged for 10 minutes (3,000 rpm). Obtained supernatant (2.5 ml) was mixed with bi-distilled water (2.5 ml) and 0.1% FeCl<sub>3</sub> solution (0.5 ml). Absorbance was measured at 700 nm. Higher absorbance indicates a higher reducing power (reducing capability).

#### **Statistical Analysis**

Statistic analysis was carried out using Statistica 6.0. (StatSoft Inc, Tulsa, OK, US). All experiments were performed at least in triplicate unless specified otherwise. Significant levels were defined at  $p < 0.05$ .

## Result and discussion

### Determination of antioxidant compounds

As a chemical structure of phenolic compounds is responsible for their antioxidant activity, the measurement of total phenolics content could be related to antioxidant properties of investigated material. Total phenolics content, total flavonoids content and total flavonoids/total phenolics ratio (TF/TP) are presented in the Table 1.

**Table 1.** Total phenolics (TP), total flavonoids (TF), total flavonoids and total phenolics ratio (TF/TP) of investigated extracts and tablets

Sample	Total phenols (mg GAE/g extract or tablet)	Total flavonoids (mg CE/g extract or tablet)	(TF/TP)x100
<b>Ehinacea dry extract</b>	30.37	18.94	62.36
<b>Mentha dry extract</b>	180.32	17.76	9.84
<b>Ginkgo dry extract</b>	66.86	9.13	13.65
<b>Tablets</b>	23.29	7.97	34.22

In all tested samples the content of total phenols was higher than the content of total flavonoids. The highest content of phenolic compounds was detected in mentha extract, 180.23 mg GAE/g. Similar content of total flavonoid compounds was observed in echinacea and mentha extracts, around 18 mg CE/g. The ratio TF/TP in echinacea extract was much higher than in the other two extracts. This could contribute to its total antioxidant activity because flavonoids are considered as the most active antioxidant phenolic compounds. Phenolics and flavonoids can play a double role in reducing the rate of oxidation, as they participate in iron chelation and trapping radicals (24).

Content of total phenolic compounds in 1 g of tablet was 23.29 mg GAE. The ratio TF/TP in tablet was around 35%, higher than in the case of mentha and ginkgo extracts.

### DPPH assay

Scavenging effect of herbal extracts and tablets on DPPH<sup>•</sup> radicals increased with increasing concentrations. At concentration of 0.01 mg/ml radical scavenging capacity of investigated extracts was higher than 90% for echinacea, around 70% for ginkgo and lower than 50% for mentha extract. In the tablet solution, concentration of 0.01mg/ml, radical scavenging capacity of DPPH radical was 18.86 %. From these results can be concluded that echinacea extracts could be considered as good antioxidant products. This can also be said for ginkgo extract as its scavenging capacity, at the same investigated concentration, was also very high.

**Table 2.** RSC (%) of tablets and ginkgo, mentha and echinacea extracts at investigated concentrations.

Concentration Absorbance	0.01 (mg/ml)	0.02 (mg/ml)	0.05 (mg/ml)
<b>Tablet</b>	18.86	33.65	86.70
<b>Ehinacea extract</b>	91.58	92.79	91.90
<b>Mentha extract</b>	31.05	65.89	99.26
<b>Ginkgo extract</b>	67.89	93.27	93.26

Radical scavenging activity was found to exhibit 50% of inhibition value ( $IC_{50}$  value) at the extract concentration of  $0.015 \pm 0.007$  mg/ml for investigated mentha extract.  $IC_{50}$  values for ginkgo and echinacea extract were lower than 0.01 mg/ml.  $IC_{50}$  for prepared tablet was 0.029 mg/ml.  $IC_{50}$  values for all investigated samples are generally very low. This indicates that all, dry extracts and investigated tablets, could be considered as good scavengers of this synthetic radical, e.g. good antioxidant compounds.

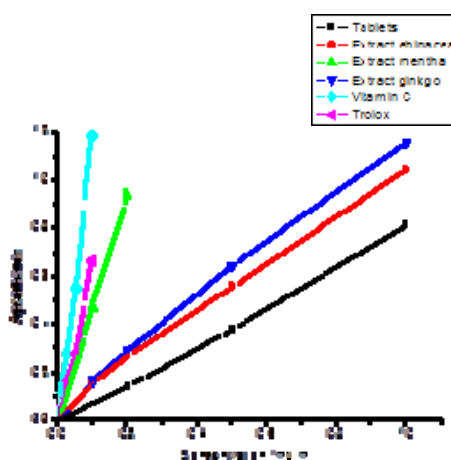
#### Determination of reducing power

Ferrous ion, which commonly exists in food systems, is well known as an effective pro-oxidant component (26). Ferrous iron has the capacity to reduce oxygen to superoxide radical, it can catalyze the decomposition of peroxide and yielding hydroxyl radical from hydrogen peroxide (27). For the measurements of the reductive ability,  $Fe^{3+} \rightarrow Fe^{2+}$  transformation, in the presence of extracts and tablets, has been investigated.

**Table 3.** Determination of investigated extracts and obtained tablets reducing power.

Concentration \ Absorbance	0.025 (mg/ml)	0.05 (mg/ml)	0.1 (mg/ml)	0.2 (mg/ml)	0.5 (mg/ml)	1.0 (mg/ml)
<b>Tablet</b>				0.136	0.375	0.813
<b>Ehinacea extract</b>			0.154	0.263	0.555	1.046
<b>Mentha extract</b>			0.469	0.937	1.953	
<b>Ginkgo extract</b>			0.162	0.287	0.641	1.156
<b>Vitamin C</b>	0.276	0.547	1.184			
<b>Trolox</b>	0.158	0.276	0.661			

Reducing power of investigated extracts and tablets was compared to reducing power of standard antioxidant compounds, Trolox and vitamin C. It is clear that both, Trolox and vitamin C, have better reductive capabilities than investigated extracts and tablets. For example at the concentration of 0.1 mg/ml, measured absorbance of vitamin C was 1.184 (Table 3). For extracts of echinacea and ginkgo, at the same concentration, it was around 0.15 (0.154 and 0.162) while for mentha extract it was almost 3 times higher than in the case of previous two extracts (0.469). Also, reducing power of mentha extract at this concentration was comparable to reducing power of synthetic antioxidant Trolox. Reducing power of prepared tablets was a few times lower than that of investigated extracts (Fig. 1)



**Fig. 1.** Reducing power of ginkgo, echinacea and mentha dried extracts and tablets on their basis and standard antioxidant compounds

EC<sub>50</sub> value (concentration needed to obtain 0.5 absorbance) for vitamin C and Trolox was 0.046 mg/ml and 0.079 mg/ml, respectively. These values are much lower than EC<sub>50</sub> values of investigated extracts and tablets. Low EC<sub>50</sub> has been determined for mentha extract, 0.107 mg/ml. In comparison to mentha extract, EC<sub>50</sub> of Echinacea and ginkgo extract were higher, 0.443 mg/ml and 0.380 mg/ml, indicating lower reducing capabilities. Highest of all measured samples was EC<sub>50</sub> of prepared tablets, 0.637 mg/ml.

Phenolic compounds, that are detected and determined in investigated extracts and tablets, can chelate pro-oxidant metal ions, thus preventing free radical formation from these pro-oxidants species (28). Phenolic compounds were detected and quantified in investigated extracts and tablets, and from the above experiment it is uphold that extract was capable of donating electrons and thus is capable of reducing iron (III).

## Conclusion

Intensive research for new, unexplored, plant antioxidant source is very significant and can bring new natural products in pharmaceutical and food industry for their every day battle with reactive oxygen species. Finding new antioxidant sources could be important for health benefit, considering many diseases that reactive oxygen species induce in biological systems. Discovering of natural sources of antioxidants could be significant also for artificial toxic antioxidants replacement in food industry.

The results of this study clearly indicated that ginkgo, echinacea and mentha extracts, as well as tablets made on their basis, are good scavengers of synthetic DPPH radicals, which indicates that they all could be used as antioxidant products. Also, they all possess reductive capabilities. Reductive capacity of investigated extracts and tablets was lower than that of investigated standard antioxidant compounds, so they could be primarily considered as antioxidants and then as products with reductive capabilities. They all are adequate sources of phenolic and flavonoid compounds, which are well known as being endowed with high antioxidant activity. All these characteristics uphold the use of tablets as dietary supplements in the case of every day free radical damage protection and prevention.

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