Antioxidative activity of *Ginkgo*, *Echinacea*, and *Ginseng* tinctures

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Key words: *antioxidant activity; Ginkgo; Echinacea; Ginseng.*

Summary. *The aim of this study was to determine the amount of phenol compounds in tinctures prepared from Ginkgo leaves, Echinacea plant, and Ginseng roots and to evaluate the antioxidative activity of these preparations. We studied the antioxidative activity using the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical cation scavenging and tyrosine nitration inhibition tests. The obtained findings showed that the amount of phenol compounds in the studied tinctures differed and ranged between 114 to 340±29 gallic acid equivalents (GAE) mg/100 mL. We found that the amount of phenol compounds in Ginkgo tincture was statistically significantly greater than that in Echinacea or Ginseng tinctures. The effectiveness of Ginkgo tincture was by 52.7% (P<0.01) lower (from 1343±11 μmol catechin/100 mL solution to 637±64 catechin/100 mL solution), compared to Echinacea tincture. Ginseng tincture was the weakest scavenger of free radicals – only 8±1 μmol catechin/100 mL solution. The inhibition of tyrosine nitration was by 34% (P<0.01) greater in Echinacea tincture, compared to Ginkgo tincture (from 892±36 μmol catechin/100 mL solution to 588±17 μmol catechin/100 mL solution). Ginseng tincture was the weakest inhibitor of tyrosine nitration – only 20±8 μmol catechin/100 mL solution, which was by 44.6 times less, compared to Echinacea tincture.*

Tests on DPPH· radical cation scavenging and inhibition of nitration showed that the antioxidative activity of Echinacea tincture was statistically significantly greater compared to that of Ginkgo or Ginseng tinctures. This allows us to conclude that antioxidative activity is determined not only by phenol compounds, but also by a complex of other components of medicinal raw material.

Introduction

The cause of coronary heart diseases, cancers, and aging processes is oxidative stress that is caused by free radicals (1, 2). One of the ways to prevent the aforementioned pathologies is application of antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species (3). Experimental studies were performed on three plants that have antioxidative properties – *Ginkgo biloba*, *Panax ginseng*, and *Echinacea purpurea* (4). *Ginkgo biloba* preparations are used in the treatment of Parkinson's and Alzheimer's diseases caused by oxidative stress. Studies have shown that oxidative stress is related to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases (5). Studies by Bulgarian researchers showed that *Panax ginseng* preparations had antioxidant activity and were used for chemotherapy prophylaxis (6). In the treatment of cancer, the human organism has to deal with cancer-combative strategies such as chemotherapy and radiation, which impede the immune defenses (7). Preparations of *Echinacea* are widely used to stimulate the immune system (8) and as alternative remedies for the prevention of common cold and infections in the upper respiratory tract (9, 10). It has been found that *Echinacea* preparations have antioxidative (4) and free radical scavenging properties (11).

The aim of this study was to determine the amount of phenol compounds in tinctures prepared from *Ginkgo* leaves, *Echinacea* plant, and *Ginseng* roots and to evaluate the antioxidative activity of these preparations.

Materials and methods

Reagents and samples

The chemicals and samples were available commercially and were used as received: tyrosine (BDH

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Chemicals, England), 3-nitrotyrosine (Aldrich), DPPH (Sigma), (+)-catechin, gallic acid, and Folin-Ciocalteu reagent (Fluka). All other chemicals used were of analytical grade.

Peroxynitrite solution was prepared according to methods indicated in literature (12). Aliquots of 0.6 M H_2O_2 (in 0.5 M $HClO_4$) and 0.5 M $NaNO_2$ (in water) (10 mL each) were pre-cooled to –2°C and mixed rapidly. The reaction was immediately quenched by addition of 5 mL –2°C cold 3.5 M NaOH. Non-reacted H_2O_2 was removed by treatment with excess of MnO_2, and the solution was filtered. The ONOO concentrations were determined spectrophotometrically in alkaline solution using ε_{302}=1670 $M^{-1}\cdot cm^{-1}$. Stock solutions were stored frozen at –80°C for several months without noticeable decomposition.

Equipment

High performance liquid chromatography (HPLC) system HP 1100 (Agilent Technologies) consisted of a quaternary pump and an autosampler, and DAD detector was used for HPLC separation; UV spectra were measured using HP 8453 (Agilent Technologies).

Inhibition of tyrosine nitration

From the 2.5 mM peroxynitrite solution in 0.05 M NaOH, a volume of 8 mL was drawn and mixed rapidly in the injector of HPLC autosampler with 42 μL 1.0 mM tyrosine solution in 0.15 M KH_2PO_4–Na_2HPO_4 buffer (pH 7.4) containing suitably diluted plant tincture. The reaction mixture was injected directly onto the HPLC column (Supelcosil ABZ+Plus 250×4.6 mm, 5 μL); the mobile phase consisted of 90% 40 mM HCOOH and 10% CH_3CN (v/v); flow rate was 1 mL/min. The chromatograms were measured at 276 nm. The activity of the tested compounds was calculated relative to the measured peak of 3-nitrotyrosine of control measurement. Quantification was done on the basis of the standard curve of catechin. Results were expressed as micromoles of catechin equivalent per 100 mL of tincture.

Measurement of DPPH quenching activity

Solution (25 μL) of plant tincture suitably dissolved in methanol was adjusted to the volume of 2.0 mL with 0.1 mM DPPH solution in methanol, and the absorbance was measured at 517 nm for 5 min after mixing. The activity of the tested compounds was calculated relative to the measured absorbance of the control measurement. Quantification was done on the basis of the standard curve of catechin. Results were expressed as micromoles of catechin equivalent per 100 mL of tincture.

Statistical analysis

Statistical analysis was performed using statistical software package Statistica 5.5. The data were presented as means ± S.E.M. Statistical analysis was performed using Student's *t* test, and $P<0.05$ was used as the level of significance.

Results and discussion

Experimental studies were performed on three tinctures: *Ginkgo* 1:5 (extrahent 70% ethanol), *Ginseng* 1:5 (extrahent 70% ethanol), and *Echinacea* 1:5 (extrahent 50% ethanol) (7, 13). According to literature data, phenol compounds in plants are the largest group of compounds with antioxidative properties (10, 14). For this reason we found it expedient to determine the total amount of phenol compounds in the investigated tinctures (Fig. 1). The total amount of phenol compounds was determined using the regression equation of the calibration curve: y=10.738x+0.061, R^2=0.98. The obtained findings showed that the amount of phenol compounds in the studied tinctures differed and ranged between 11±4 to 340±29 GAE mg/100 mL.

Further on, we studied the antioxidative activity of *Ginkgo*, *Echinacea*, and *Ginseng* tinctures, using the standard DPPH radical cation scavenging and tyrosine nitration inhibition tests. Total antioxidant capacity assay, such as DPPH· method, is most common for antioxidant activity for large-scale examination (1). We found that an antioxidant releases hydrogen, thus inactivating free radicals that consequently become stable compounds of DPPH-H type (1). Our findings showed that *Echinacea* tincture was most effective in scavenging DPPH radicals (Fig. 2). The effectiveness of *Ginkgo* tincture was by 52.7% ($P<0.01$) lower (from 1343±11 μmol catechin/100 mL solution to 637±64 μmol catechin/100 μL solution) compared to *Echinacea* tincture. *Ginseng* tincture was the

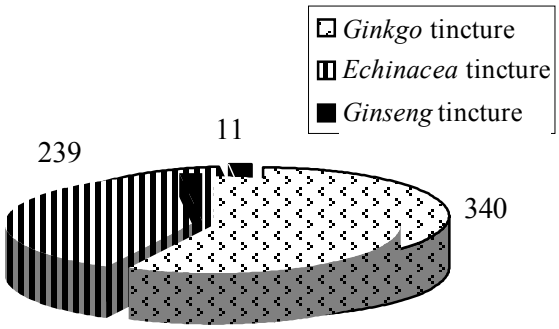

Fig. 1. **The amount of phenol compounds (GAE mg/100 mL) in the investigated tinctures (n=5)**

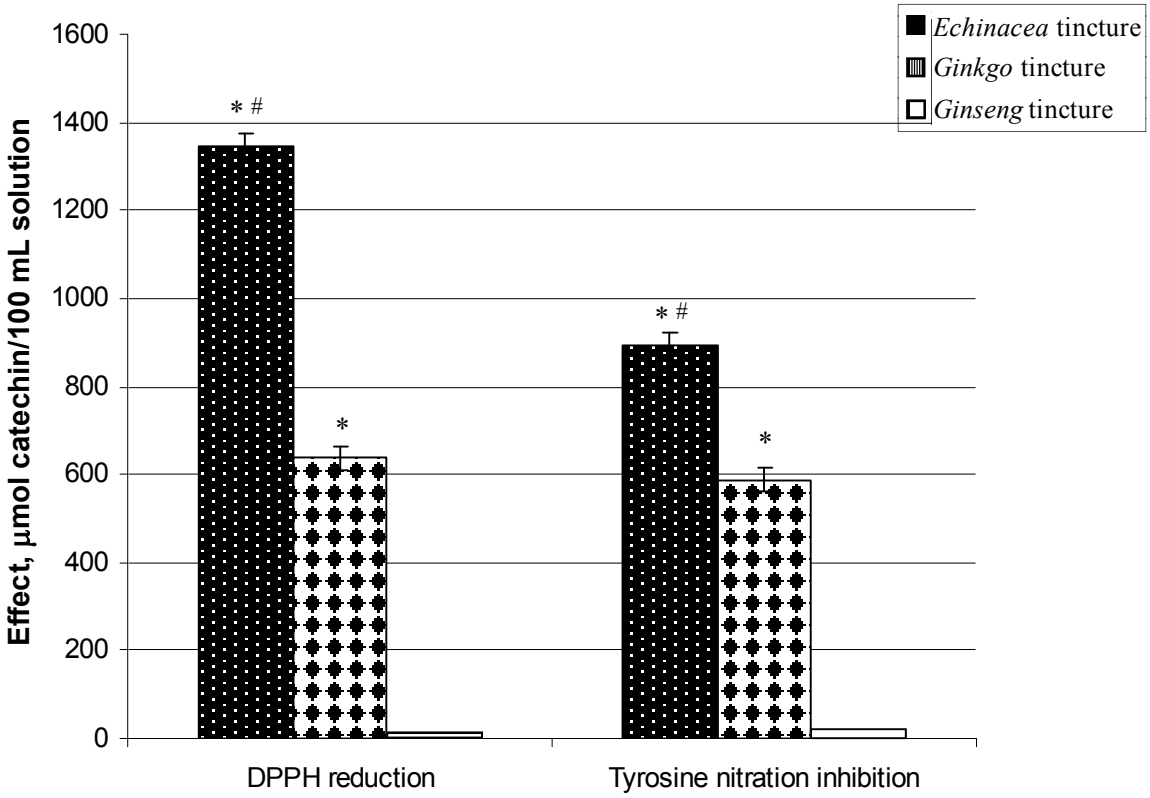

Fig. 2. **Antioxidative activity of *Echinacea*, *Ginkgo*, and *Ginseng* tinctures, determined using DPPH and tyrosine nitration techniques (n=6)**

*P<0.01 *vs. Ginseng* tinctures; #P<0.01 *vs. Ginkgo* tinctures.

weakest scavenger of free radicals – only 8±1 µmol catechin/100 mL solution.

Peroxynitrite is a potent nitrating and oxidizing agent that is formed by a rapid reaction of nitric oxide with superoxide anion. Nitric oxide is a simple inorganic radical exhibiting diverse physiological functions, including the regulation of neurotransmission and vascular tone (12, 15). Peroxynitrite, a biological toxin, produced *in vivo* by the nearly diffusion-controlled reaction of nitrogen monoxide with superoxide, can nitrate and oxidize various biomolecules, such as thiols, lipids, carbohydrates, and nucleic acids (16). The inhibition of tyrosine nitration was by 34% (P<0.01) greater in *Echinacea* tincture, compared to *Ginkgo* tincture (from 892±36 µmol catechin/100 mL solution to 588± 17 µmol catechin/100 mL solution). *Ginseng* tincture was the weakest inhibitor of tyrosine nitration – only 20±8 µmol catechin/100 mL solution, which was by 44.6 times lower, compared to *Echinacea* tincture.

According to literature, there is a proven relationship between antioxidant activity and total phenolic content in herbs, vegetables, and fruits (1, 17). The results of our study showed that *Ginkgo* tincture had the greatest amount of phenol compounds, and *Echinacea* tincture had the strongest properties of free radical scavenging and nitration inhibition.

The study showed that tinctures produced from all three investigated plants – *Echinacea purpurea* plant, *Ginkgo biloba* leaves, and *Panax ginseng* roots – had antioxidative activity. The obtained findings allow estimation of the ability of these tinctures to inactivate free radicals. Although the greatest amount of phenol compounds was found in *Ginkgo biloba* tincture, *Echinacea* tincture had the greatest antioxidative activity. This allows stating that antioxidative activity is not directly related to the amount of phenol compounds detected in plants.

Conclusions

1. We found that the amount of phenol compounds in *Ginkgo* tincture was statistically significanty greater than that in *Echinacea* or *Ginseng* tinctures.

2. Tests on DPPH radical cation scavenging and inhibition of nitration showed that the antioxidative activity of *Echinacea* tincture was statistically significantly greater compared to that of *Ginkgo* or *Ginseng* tinctures. This allows us to conclude that antioxidative activity is determined not only by phenol compounds, but also by a complex of other components of medicinal raw material.

Ginkmedžių, ežiuolių ir ženšenių tinktūrų antioksidacinis aktyvumas

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Raktažodžiai: antioksidacinis aktyvumas, ginkmedis, ežiuolė, ženšenis.

Santrauka. *Darbo tikslas*. Nustatyti fenolinių junginių kiekį tinktūrose, pagamintose iš ginkmedžių lapų, rausvažiedžių ežiuolių žolės bei ženšenių šaknų, ir įvertinti šių preparatų antioksidacinį aktyvumą. Antioksidacinį aktyvumą tyrėme naudodami modelinius 2,2-difenil-1-pikrilhidrazilo (DPPH·) radikalų katijono sujungimo ir tirozino nitrinimo inhibicijos testus. Gauti rezultatai parodė, kad fenolinių junginių kiekis tirtose tinktūrose skiriasi. Jis svyravo nuo 11±4 iki 340±29 GAE (galo rūgšties ekvivalentai) mg/100 ml. Nustatėme, kad ginkmedžių tinktūroje fenolinių junginių kiekis yra statistiškai reikšmingai didesnis nei ežiuolių ar ženšenių tinktūrose. Ginkmedžių tinktūros antioksidacinis efektyvumas buvo 52,7 proc. ($p<0,01$) mažesnis (nuo 1343±11 μmol katechino/100 ml tirpalo iki 637±64 μmol katechino/100 ml tirpalo) palyginti su ežiuolių tinktūra. Silpniausia radikalų gaudyklė buvo ženšenių tinktūra – tik 8±1 μmol katechino/100 ml tirpalo. Tirozino nitrinimo inhibicija buvo 34 proc. ($p<0,01$) didesnė ežiuolių ekstrakte palyginti su ginkmedžių (nuo 892±36 μmol katechino/100 ml tirpalo iki 588±17 μmol katechino/100 ml tirpalo). Mažiausia tirozino nitrinimo inhibicija pasižymėjo ženšenių tinktūra – tik 20±8 μmol katechino/100 ml tirpalo ir tai buvo 44,6 kartus mažiau palyginti su ežiuolių tinktūra.

DPPH· radikalų katijono sujungimo ir nitrinimo inhibicijos testai parodė, kad ežiuolių tinktūros antioksidacinis aktyvumas statistiškai reikšmingai didesnis palyginti su ginkmedžių ar ženšenių tinktūromis. Taigi galima daryti išvadą, kad antioksidacinį aktyvumą lemia ne tik fenoliniai junginiai, bet ir kompleksas kitų medžiagų, įeinančių į vaistinės žaliavos sudėtį.

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