Antioxidant activity of ginger extract and identification of its active components

Silvia Mošovská[a], Dominika Nováková[a], Michal Kaliňák[b]

[a]*Department of Nutrition and Food Assessment, Faculty of Chemical and Food Technology, Radlinského 9, 812 37 Bratislava, Slovakia*
[b]*Department of NMR Spectroscopy and Mass Spectrometry, Faculty of Chemical and Food Technology, Radlinského 9, 812 37 Bratislava, Slovakia*
silvia.mosovska@stuba.sk

Abstract: The object of presented study was the evaluation of antioxidant activity of extract from *Zingiber officinale*. A method for the preparation of the extract was chosen, which enabled to isolate mostly phenolic compounds. According to the measurements with Folin-Ciocalteu reagent, obtained extract comprised total polyphenols (181.41 mg_{GAE}/g of extract) from which flavonoids contributed to 7.8 % (14.15 $mg_{quercetin}$/g of extract). Nuclear magnetic resonance (NMR) analysis showed that the phenolic ketones were predominant in the extracts of whole phenolic compounds. According to results of two spectrophotometric methods (ABTS and DPPH test), ginger extract showed stronger ability to scavenge DPPH radical than ABTS cation radical.

Keywords: ginger, phenols and flavonoids, NMR analysis, antioxidant activity

Introduction

Ginger (*Zingiber officinale* Rosco) a member of the family Zingiberaceae, is well-known as a spice which has been used for over 2000 years (Stoilova et al., 2007; Hasan et al., 2012). This species contains biological active constituents including terpenes and oleoresin. From terpene, the major identified components are sesquiterpene hydrocarbons and phenolic compounds such as gingerol and shogaol. Gingerols known as phenolic ketones can be converted to shogaols, zingerone, and paradol (Rahmani et al., 2014) which produce the "hot" sensation in the mouth (Aly et al., 2013).

Thanks to the low toxicity of ginger and its broad spectrum of biological and pharmacological applications, it has been increasingly used (Aly et al., 2013). Ginger has been used as a refrigerant, astringent and flavouring agent, and as a digestive in medicine. Rhizome paste has been traditionally applied for improving the healing of wounds, cuts and antipruritic (Srivastava et al., 2006). Its broad spectrum of biological activities includes antioxidant, antimicrobial, antitumor or anti-diabetic effects.

Masuda et al (2004) identified more than 50 compounds including gingerols or diarylheptanoids obtained from ginger with antioxidant activity. The ginger causes the suppression of both cyclooxygenase and lipoxygenase metabolites and arachidonic acid (Dugasani, 2010). According to Li (2011), shogaol and [6]-dehydroshogaol has been able to inhibit NO production in LPS stimulated macrophages.

Ginger extract has showed antimicrobial activity against broad spectrum of pathogenic microorganisms such as *Escherichia coli, Salmonella typhi, Bacillus subtilis* and yeast *Candida albicans* (Mahady, 2003; Azu, 2007). Main components, including [6]-gingerol and [12]-gingerol, isolated from ginger rhizome displayed antibacterial activity against periodontal bacteria (Park, 2008). Hiserodt (1998) mentioned in his research that [10]-gingerol is active inhibitor of *Micobacterium avium and M. tuberculosis*.

Except for antioxidant and antimicrobial effects of phenolic compounds contained in *Zingiber officinale*, these components also have anti-carcinogenic and anti-mutagenic activities (Khader, 2010). According to Lee (2008), [6]-gingerol inhibits cell adhesion, invasion, motility and activities of human breast cancer cell lines.

The purpose of this study was to investigate the antioxidant activities of the ginger extract with regard to the free radicals 2,2-diphenyl-1-picryl hydrazyl and ABTS cation radical, respectively. Qualitative analysis of main components of extract and the amount of polyphenolic compounds, including flavonoids, was also carried out.

Materials and methods

Plant material

Ginger powder BIO (*Zingiber officinale*) was bought in local shops. Best before: 07. 2016; Lot number: SO14073013.

Preparation of extract

The powdered root of ginger (100 g) was percolated using 500 ml of methanol at room temperature for 72 h. After percolation, this percolate was filtered

by Büchner funnel and then it was evaporated to dryness in a rotary evaporator at temperature lower than 40 °C. The residue was dissolved in ethyl acetate. Then, the water was added at ratio 1 : 1 (v/v) and mixture was mixed for 2 minutes (2×). Subsequently, mixture was separated by separation funnel into two phases (ethyl acetate and water phases). Ethyl acetate phase was concentrated by a rotary evaporator at temperature lower than 40 °C. Obtained extract was stored at temperature lower than 5 °C. For analysis, ginger extract was dissolved in methanol.

Determination of total phenolic compounds and flavonoid content

The total polyphenol content of the ginger extract was evaluated by Folin-Ciocalteu reagent according to Yu et al. (2004). Their concentration in extract was expressed in mg of gallic acid equivalents (mg_{GAE}) per 1 g of extract.

The flavonoid content was determined according to Kreft et al. (2002). The results were expressed as mg of quercetin equivalents ($mg_{quercetin}$) per 1 g of extract.

Determinations of main constituents of ginger extract using NMR analysis

Qualitative analysis of main constituents of ginger extract was measured using nuclear magnetic resonance (NMR) according to Kaliňák et al. (2014). Briefly, ^{1}H-NMR spectra were run on a 600 MHz NMR spectrometer (Agilent) equipped with indirect detection probe. Deuterated methanol was used as solvent. The resulting ^{1}H-NMR spectra were processed using the MESTRENOVA program.

Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

The radical scavenging ability was determined as described by Yen et al. (1995). Briefly, 1 ml from methanolic solution of DPPH was added to 1 ml from the samples with different concentrations of ginger extract and 3 ml of methanol. The samples were kept at room temperature in the dark and after 30 min the optic density was measured at 517 nm. The antioxidant activity of ginger extract was expressed by IC_{50} value (mg/ml).

Evaluation of radical scavenging potential by ABTS radical cation assay

The free radical scavenging activity of ginger extract was determined using ABTS radical cation decolourization assay according to Arts et al. (2004). Briefly, 0.05 ml from samples with different concentrations of ginger extract was added to 2 ml from ABTS cation radical. The samples were kept at room temperature in the dark and after 10 min the optic density was measured at 730 nm. The antioxidant activity of ginger extract was expressed by IC_{50} value (mg/ml).

Statistical analysis

Experiments were carried out in duplicate and expressed as mean ± standard deviation (SD).

Results and discussion

Determination of total phenolic compounds and flavonoids content

The phenolic compounds represent one of the main groups of secondary metabolites in plants and they are present in foods and nutraceutics. These phytochemicals have a wide range of biological actions, including the ability to act as antioxidants, ameliorate inflammation, modulate enzyme activity, and regulate gene expression (McKay, 2015). In present study, total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalent (GAE). As shown in the Table 1, the content of total polyphenolics in ginger extract was in amount of 181.41 mg_{GAE}/g ginger extract. This value is significantly lower in compared to research of Stoilova et al. (2007). Mentioned authors stated polyphenolic content in ginger extract nearly 5 times (871 mg_{GAE}/g ginger extract) higher than our sample. As Jaffery (2003) stated in his study, the composition and quantity of the phenolic are vary significantly according to different intrinsic and extrinsic factors, including plant genetics and cultivars, soil and growing conditions, maturity state and harvest conditions. Extracted process also has a significant effect on the composition and properties of the final extract (Gallardo et al., 2006).

The largest part of the polyphenolic compounds is represented by flavonoids. These components

Tab. 1. Comparison of total phenolics and flavonoids in ginger extract.

	Total phenolics (mg_{GAE}/g ginger extract)	Flavonoids ($mg_{quercetin}$/g ginger extract)
Ginger extract	181.41 ± 0.07	14.15 ± 0.12

Results are means ± SD (n = 2).

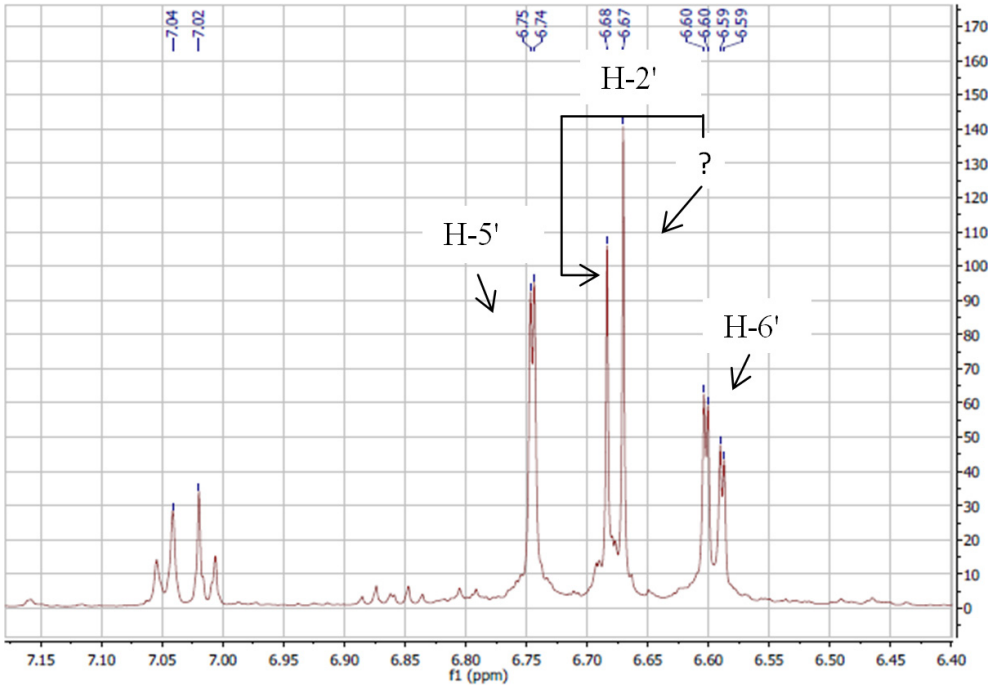

Fig. 1. Section of ^{1}H-NMR standard spectra. Numbers correspond to ^{1}H positions as presented in Fig. 2.

were analysed spectrophotometrically according to the method with $AlCl_3$ and calculated as quercetin equivalent. In compared to total phenolics, flavonoid amounts were estimated in amount of 14.15 $mg_{quercetin}$/g ginger extract (Table 1). Previous argument of influence of various factors on biological active compound content has also confirmed by comparing of flavonoid amount in our sample and studies of other authors. Pawar et al. (2011) have found that the flavonoid content in ginger extract was in the range from 1.3 to 3.8 $mg_{quercetin}$/g ginger extract.

Determinations of main constituents of ginger extract using NMR analysis

Determinations of main constituents of studied extract were done by NMR analysis according to Kaliňák et al. (2014). ^{1}H-NMR standard spectra were measured. Spectrophotometric method with Folin-Ciocalteu reagent suggested that obtained extract has contained polyphenolic compounds. This result was confirmed with NMR analysis too (Fig. 1).

According to measured spectra and compared to spectra in literature (Wu, 2007), phenolic ketones have been represented among isolated compounds, including gingerols, shogaols, paradols and gingerdions (Fig. 2).

Because these compounds are structurally similar, it cannot be determined with certainty what kind of component is exactly one according to chemical shift. For an exact description of these substances, more detailed analysis is needed on which we will focus in the future.

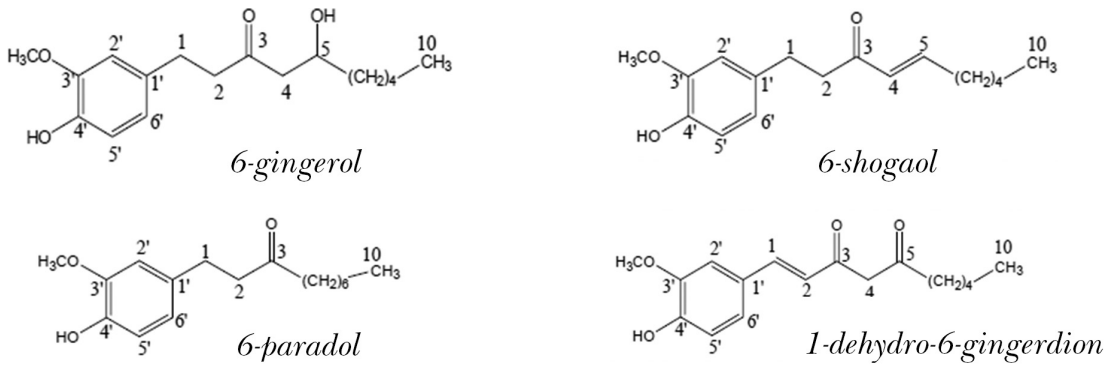

Fig. 2. Chemical structure of possible compounds (Wu, 2007) occurring in tested ginger extract.

Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) and ABTS cation radical

Oxidation of biological molecules induces a variety of pathological disease including atherosclerosis or cancer. These damages are caused due to the presence of free radicals. For that reason, the concept of pharmacological supplements to defend against free radicals with antioxidants has become an intense area of research (Gounder and Lingmallu, 2012). According to Atashak (2014), [6]-gingerol, [6]-shogaol have displayed strong antioxidant activity *in vitro*.

It is known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests used to prove the ability of the components of the ginger extract to act as donors of hydrogen atoms (Stoilova et al., 2007). The obtained results are presented in Tab. 2. The ginger extract showed antioxidant effect in inhibiting DPPH radical, IC_{50} was 4.25 mg/ml.

However, tested extract exhibited excellent free radical scavenging properties against ABTS cation radical (Tab. 2) if compared to DPPH radical scavenging potential. IC_{50} was 0.4 mg/ml. Different results of IC_{50} value between tested methods was probably caused unequal structure and mechanism of action of both molecules as ABTS cation radical as DPPH radical. DPPH reactions are highly sensitive to reaction environment, i.e. water and solvent, pH, oxygen, light exposure (Schaich, 2015). ABTS radical cation is reactive to most antioxidants (Cano et al., 2000). This radical reacts with antioxidant relatively rapidly. On the contrary, the DPPH test is based on the capability of DPPH radical to react with H-donors including phenolics (Martysiak-Žurovska and Wenta, 2012). DPPH radical is likely more selective than ABTS radical cation in reaction with H-donors (Schaich, 2015). DPPH radical does not react with flavonoids, which contain no OH-groups in B-ring as well as with aromatic acids containing only one OH-group (Roginsky and Lissi, 2005).

Tab. 2. Scavenging effects of ginger extract on DPPH radical and ABTS radical cation.

Methods of antioxidant activity determination		
	DPPH test	**ABTS test**
IC 50 (mg/ml)	4.25 ± 0.07	0.40 ± 0.014

Results are means ± SD (n = 2).

Conclusions

Data in this paper indicated that ginger extract is good source of polyphenolic compounds, including gingerols, shogaols, paradols and gingerdions. It manifested a very good scavenging of ABTS radical cation and DPPH radical, respectively. These obtained results suggest potential of ginger extract as an additive in the food and pharmaceutical industries.

Acknowledgments

This work was supported by the Slovak Grant Agencies VEGA (Grant No. 1/0495/13 and 1/0980/15) and by VMSP-II-0024-09 and the Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic for the Structural Funds of EU, OP R&D of ERDF in the frame of the Project "Evaluation of natural substances and their selection for prevention and treatment of lifestyle diseases" (ITMS 26240220040),

References

Aly UI, Abbas MS, Taha HS, Gaber ESI (2013) Glob J Bot Sci 1: 9–17.

Arts MJTJ, Haenen GRMM, Voss H, Bast H (2004) Food Chem 42: 45–49.

Atashak S, Peeri M, Azarbayajani MA, Stannard SR (2014) J Exerc Sci Fit 12: 26–30.

Azu N, Onyeagba R (2007) J Trop Med 3: 1–10.

Cano A, Acosta M, Arnao MB (2000) Redox Rep 5: 365–370.

Dugasani S, Pichika MR, Balijepalli MK, Tandra S, Korlakunta JN (2010) J Ethnopharmacol 127: 515–520.

Gallardo C, Jimenéz L, García-Conesa MT (2006) Food Chem 99: 455–463.

Gounder DK, Lingmallu J (2012) Ind Crop Prod 38: 124–131.

Hasan HA, Raauf AMR, Abd Razik BM, Hassan BAR (2012) Pharmaceut Anal Acta 3: 2153–2435.

Hiserodt RD (1998) J Agricult Food Chem 46: 2504–2508.

Jaffery EH, Brown AF, Kurilich AC, Keck AS, Matusheski N, Klein BP, Juvik JA (2003) J Food Compost Anal 16: 323–330.

Kaliňák M, Šimkovič M, Žemla P, Maťaťa M, Molnár T, Liptaj T, Varečka L, Hudecová D (2014) FEMS Microbiol Lett 357: 201–207.

Khader M, Bresqen N, Eckl PM (2010) J Ethnopharmacol 127: 319–324.

Kreft I, Fabjan N, Yasumoto K (2002) Food Chem 98: 508–512.

Lee HS, Seob EY, Kangc NE, Kim WK (2008) J Nutr Biochem 19: 313–319.

Li F, Wang Y, Parkin KL, Nitteranon V, Liang J, Yang W, Li Y, Zhang G, Hu Q (2011) Food Res International 44: 1597–1603.

Mahady GB, Pendland SL, Yun GS, Lu ZZ, Stoia A (2003) Anticancer Res 23: 3699.

Martysiak-Žurovska D, Wenta W (2012) Acta Sci Technol Aliment 11: 83–89.

Masuda Y, Kikizaki H, Hisamoto M, Nakatani N (2004) Biofactors 21: 293–296.

McKay DL, Chen CY, Zampariello CA, Blumberg JB (2015) Food Chem 168: 233–240.

Park M, Bae J, Lee DS (2008) Phytother Res 22: 1446–1449.
Pawar N, Pai S, Nimbalkar M, Dixit G (2011) Food Chem 126: 1330–1336.
Rahmani AH, Al shabrmi FM, Aly SM (2014) Int J Physiol Patophysiol Pharmacol 6: 125–136.
Roginsky V, Lissi EA (2005) Food Chem 92: 235–254.
Schaich KM, Tian X, Xie J (2015) J Funct Foods 14: 111–125.
Srivastava S, Srivastava M, Rawat AKS, Mehrotra S (2006) Proc Natl Acad Sci 76: 153–160.
Stoilova I, Krastanov A, Stoyanova A (2007) Food Chem 102: 764–770.
Wu SC, Yen GC, Wang BS, Chiu CK, Yen WJ, Chang LW, Duh PD (2007) Food Sci Technol 40: 506–512.
Yen GC, Chen HY (1995) J Agricult Food Chem 43: 27–32.