

Inhibitory effects of aqueous extract of two varieties of ginger on some key enzymes linked to type-2 diabetes in vitro

GANIYU OBOH – AYODELE JACOBSON AKINYEMI –
ADEDAYO OLUWASEUN ADEMILUYI – STEPHEN ADENIYI ADEFEGHA

Summary

Management of the blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of saccharide hydrolysing enzymes have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type-2 diabetes mellitus. This study compares the phenolic contents and antioxidant properties of two varieties of ginger, namely, Red (*Zingiber officinale* var. *Rubra*) and white (*Zingiber officinale* Roscoe), and their inhibitory effects on the activities of α -amylase and α -glucosidase (key enzymes linked to type-2 diabetes) using an in vitro model. The results show that the aqueous extracts of red ginger had significantly ($P < 0.05$) higher phenolic contents as well as higher antioxidant activities than the aqueous extracts of white ginger. However, contrary to the phenolic contents and antioxidant properties, white ginger had a significantly ($P < 0.05$) stronger inhibitory effect on α -amylase and α -glucosidase activities than red ginger. Furthermore, both extracts exhibited mild α -amylase and stronger α -glucosidase inhibitory activities, suggesting their potential use in dietary intervention in the management or control of postprandial hyperglycemia associated with type-2 diabetes. White ginger exhibited stronger enzyme inhibitory effects than red ginger.

Keywords

Zingiber officinale; type-2 diabetes; α -amylase; α -glucosidase; inhibition; antioxidant

Hyperglycemia is a condition associated with diabetes mellitus and is linked to most diabetes complications as their primary cause. Hyperglycemia is a condition of abnormal rise in plasma glucose level, and in type-2 diabetes is a result of insulin insensitivity. Prolonged hyperglycemia leads to increased generation of reactive oxygen species (ROS) and alteration of endogenous antioxidants. Postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins and biomolecules; resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment or management of diabetes mellitus, in particular type-2 diabetes, and in reducing chronic vascular complications [1]. Inhibition of enzymes involved in the digestion of polysaccharides, such as α -amylase and α -glucosidase, is one of the therapeutic approaches for managing or controlling hyperglycemia [2].

Saccharides are the major constituents of human diet and polysaccharides belong to main components that mainly play a role in the energy supply. The dietary saccharides are first broken down to monosaccharides by certain gastrointestinal enzymes, since only monosaccharides can be absorbed from intestinal lumen [3, 4]. α -Amylase degrades complex dietary saccharides to oligosaccharides and disaccharides that are ultimately converted into monosaccharides by α -glucosidase. Liberated glucose is then absorbed by the gut [5]. The inhibition of enzymes involved in the digestion of saccharides may significantly reduce the postprandial increase of blood glucose thus delaying the process of saccharide hydrolysis and absorption [4]. Several reports have been published on established enzyme inhibitors such as acarbose, miglitol, voglibose, nojirimycin and 1-deoxynojirimycin and their favorable effects on blood glucose levels after food uptake [6]. Enzyme inhibitors may

Ganiyu Obboh, Ayodele Jacobson Akinyemi, Adedayo Oluwaseun Ademiluyi, Stephen Adeniyi Adefegha, Department of Biochemistry, School of Sciences, Federal University of Technology, P. M. B. 704, 340001 Akure, Nigeria.

Correspondence author:

Ganiyu Obboh, e-mail: goboh2001@yahoo.com

also act as effective antiobesity agents [7]. This attribute could be due to inhibition of saccharide assimilation, through inhibiting starch breakdown. With the reduced amount of amylase available for the breakdown, complex saccharides have a better chance of travelling through the gastrointestinal tract (GIT) without being assimilated, and are eventually excreted from the body instead of being converted into storage fat.

Plants have been used ethno-medicinally in the treatment of diabetes for several centuries. In recent years, research on medicinal plants for the management of diabetes has attracted the interest of scientists [8]. A number of plants are known to exert their anti-hyperglycemic activity via inhibition of saccharide hydrolysing enzymes. Natural enzyme inhibitors from plant sources have offered an attractive strategy for the control of postprandial hyperglycemia [9].

Ginger (*Zingiber officinale*) is cultivated in the tropics for its edible rhizome which serves a variety of purposes including culinary and medicinal applications [10]. The rhizome is a widely used spice and functional food. Ginger has been an important ingredient in Oriental herbal medicine.

Medicinal properties attributed to ginger include anti-arthritis [11], anti-migraine [12], anti-inflammatory [13], hypolipidemic [14], hypocholesterolemic [14] and anti-nausea properties [10]. Ginger is known to contain a number of potentially bioactive substances, mainly gingerols and their related dehydration products, shogaols, as well as volatile oils including sesquiterpenes and monoterpenes [15, 16]. Studies on hypoglycemic properties of ginger in animals have been published [17]. SHARMA and SHUKLA [18] reported a small but significant blood glucose-lowering effect of ginger juice in diabetic and non-diabetic animals. MASCOLO et al. [19] reported a significant hypoglycemic activity in normal rabbits at different times under a variety of administration schedules and doses. AKHANI et al. [20] also observed that ginger juice exhibited hypoglycemic activity in both normal and streptozotocin-induced diabetic rats. Although some in vivo studies have been reported on the antidiabetic properties of *Zingiber officinale*, limited information is available on the possible mechanism by which *Zingiber officinale* renders its hypoglycemic properties. In view of this, the present study seeks to compare the inhibitory effects of the water extractable phytochemicals of red ginger (*Zingiber officinale* var. Rubra) and white ginger (*Zingiber officinale* Roscoe) on key enzymes linked to type-2 diabetes *vis a vis* their phenolic contents and antioxidant properties.

MATERIALS AND METHODS

Materials

Fresh samples of *Zingiber officinale* Roscoe and *Zingiber officinale* var. Rubra were purchased at the Erekesan market in Akure metropolis, Nigeria. Authentication of the samples was carried out at the Department of Biology, Federal University of Technology, Akure, Nigeria. All the chemicals used were of analytical grade.

Aqueous extract preparation

The inedible portions of the rhizomes were removed from the edible portions. The edible portions were subsequently washed in distilled water, chopped into small pieces by table knife, air-dried and milled. An amount of 5 g of the milled sample was soaked in 100 ml distilled water for about 24 h, the mixture was filtered and later centrifuged at 358 g for 10 min to obtain a clear supernatant which was then used for subsequent analysis [21].

α -Amylase inhibition assay

The aqueous extracts dilution (500 μ l) and 500 μ l of 0.02 mol·l⁻¹ sodium phosphate buffer (pH 6.9 with 0.006 mol·l⁻¹ NaCl) containing hog pancreatic α -amylase (EC 3.2.1.1; 0.5 mg·ml⁻¹) were incubated at 25 °C for 10 min. Then, 500 μ l of 1% starch solution in 0.02 mol·l⁻¹ sodium phosphate buffer (pH 6.9 with 0.006 mol·l⁻¹ NaCl) was added to the reaction mixture. Thereafter, the reaction mixture was incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid (DNSA). The mixture was then incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance was measured at 540 nm in a UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom) [22].

α -Glucosidase inhibition assay

Appropriate dilution of the aqueous extracts (50 μ l) and 100 μ l of α -glucosidase solution (1.0 U·ml⁻¹) was incubated at 25 °C for 10 min. Thereafter, 50 μ l of 5 mmol·l⁻¹ *p*-nitrophenyl- α -D-glucopyranoside solution in 0.1 mol·l⁻¹ phosphate buffer (pH 6.9) was added. The reaction mixture was then incubated at 25 °C for 5 min, and then absorbance was measured at 405 nm in the spectrophotometer. The α -glucosidase inhibitory activity was expressed as percentage inhibition [23].

Determination of total phenol contents

Total phenol contents were determined in the extracts using the method of SINGLETON et al. [24].

Appropriate dilutions of the extracts were oxidized with 2.5 ml of 10% Folin–Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated using gallic acid as a standard.

Determination of total flavonoid content

The total flavonoid contents of the extracts was determined using a slightly modified method of MEDA et al. [25]. Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 μ l of 10% AlCl_3 , 50 μ l of 1 mol·l⁻¹ potassium acetate and 1.4 ml water, and was incubated at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was measured at 415 nm in the spectrophotometer. Total flavonoid content was calculated using quercetin as a standard.

DPPH free radical-scavenging ability

The free radical-scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated as described by GYAMFI et al. [26]. Briefly, an appropriate dilution of the extracts (1 ml) was mixed with 1 ml of 0.4 mmol·l⁻¹ methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm in the spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference, which contained all the reagents without the test sample.

Reducing activity

The reducing activity of the ginger extracts was determined by assessing the ability to reduce FeCl_3 solution as described by PULIDO et al. [27]. A 2.5 ml aliquot was mixed with 2.5 ml of 200 mmol·l⁻¹ sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 ml of 10% trichloroacetic acid was added. This mixture was then centrifuged at 805 g for 10 min. A volume of

5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm in the spectrophotometer and the ferric reducing antioxidant power (FRAP) was subsequently calculated as an equivalent of ascorbic acid.

Data analysis

Replicate results were pooled and expressed as mean \pm standard deviation (SD) [28]. A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were calculated. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

First, the ability of the ginger extracts to inhibit α -amylase activity in vitro was investigated and the results are presented in Fig. 1. The results revealed that both extracts inhibited α -amylase in a dose-dependent manner (in the range of 0–3 mg·ml⁻¹). However, judging by the IC_{50} (extract concentration causing 50% enzyme inhibition) values (Tab. 1), white ginger had a slightly higher inhibitory activity than red ginger. Nevertheless, there was no significant difference ($P > 0.05$) in the α -amylase inhibitory activity of both ginger varieties. The determined α -amylase inhibitory activity agreed with some earlier reports where plant phytochemicals from green and black tea inhibited saliva α -amylase activity [29] and inhibitory effects of *Allium* spp. on α -amylase activity [30].

Furthermore, the ability of the ginger extracts to inhibit α -glucosidase activity in vitro was investigated and the results are presented in Fig. 2. Both extracts exhibited a dose-dependent enzyme inhibitory activity in the range of 0–2 mg·ml⁻¹. However, white ginger exhibited significantly ($P < 0.05$) higher inhibitory activity than the red ginger, when taking into account the IC_{50} values of the aqueous extracts (Tab. 1). The results of the enzyme (α -amylase and α -glucosidase) inhibitory assays did not agree with the phenolic contents and antioxidant activities of both ginger extracts: white ginger with lower phenolic content and lower antioxidant

Tab. 1. Total phenolic contents, flavonoid contents and IC_{50} of the enzyme inhibitory activities of aqueous extracts of ginger varieties.

Samples	Total phenol content [mg·g ⁻¹]	Total flavonoid content [mg·g ⁻¹]	IC_{50} of the enzyme inhibitory activity [mg·ml ⁻¹]	
			α -amylase	α -glucosidase
White ginger	0.62 \pm 0.09	0.35 \pm 0.01	3.14 \pm 0.05	1.68 \pm 0.04
Red ginger	0.95 \pm 0.08	0.54 \pm 0.00	3.51 \pm 0.12	2.01 \pm 0.05

Values represent mean \pm standard deviation, $n = 3$.

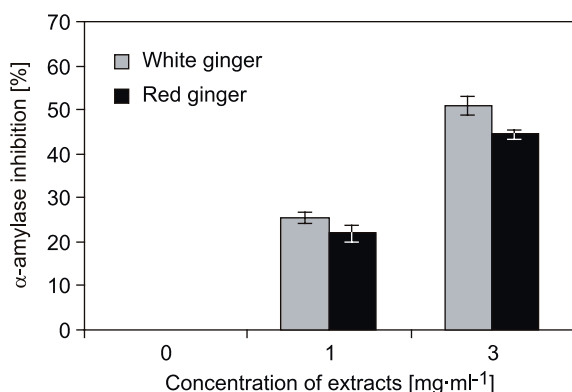


Fig. 1. α -Amylase inhibitory activities of aqueous extract of ginger varieties. Values represent mean \pm standard deviation, $n = 3$.

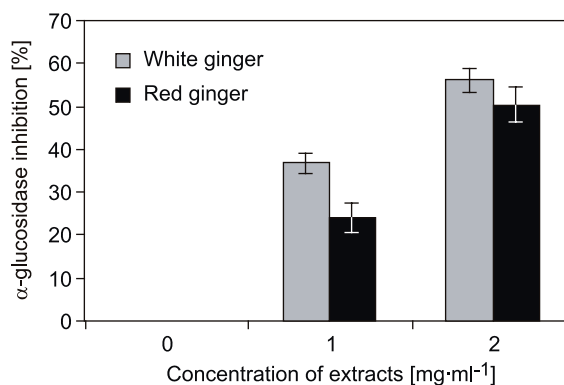


Fig. 2. α -Glucosidase inhibitory activities of aqueous extract of ginger varieties. Values represent mean \pm standard deviation, $n = 3$.

activity had higher enzyme (α -amylase and α -glucosidase) inhibitory activities. This higher inhibitory activities could be attributed to the presence of some non-phenolic phytochemicals that are potent enzyme inhibitors, exhibiting an additive or synergistic effect with the phenolics present in white ginger. This may be due to the heterogeneous nature of the aqueous extracts.

In addition, the α -glucosidase inhibitory activities of both ginger extracts were significantly ($P < 0.05$) higher than their α -amylase inhibitory activities. This is in agreement with earlier reports showing that plant phytochemicals are mild inhibitors of α -amylase and strong inhibitors of α -glucosidase activities [31]. This property may confer advantage over synthetic drugs in the management of postprandial blood glucose, such as Acarbose, which strongly inhibit α -amylase. Stronger inhibition of α -glucosidase activity and mild inhibition of α -amylase activity of the ginger extracts could address the major drawback of currently used α -glucosidase and α -amylase inhibitory drugs with side effects such as abdominal distention, flatulence, meteorism and possibly diarrhea [32]. It has been suggested that such adverse effects might be caused by the excessive pancreatic α -amylase inhibition resulting in the abnormal bacterial fermentation of undigested saccharides in the colon [32, 33]. Therefore, this study buttress the claim that natural inhibitors from dietary plants have lower inhibitory effect against α -amylase activity and stronger α -glucosidase inhibitory activity, and could be effectively used in the therapy of postprandial hyperglycemia with minimal side effects [31]. This finding agrees with the previously published properties of egg plant phenolics, which have been recommended as a choice diet for the management of type 2 diabetes [34].

The results on the total phenol contents and the total flavonoid contents of the aqueous extract of both red ginger and white ginger are shown in Tab. 1. The results revealed that the total phenol content of the red ginger extract was significantly ($P < 0.05$) higher than that of the white ginger extract. The basis for the significant difference ($P < 0.05$) in the total phenolics could result from the fact that red ginger contains anthocyanins and tannin besides gingerols and shogaols [35]. Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals, they may chelate metallic catalysts, activate antioxidant enzymes, reduce α -tocopherol radicals and inhibit oxidases [36].

Furthermore, the flavonoid contents were significantly ($P < 0.05$) higher in red ginger extracts than in white ginger extracts (Tab. 1). The trend in the total flavonoid contents agreed with the total phenolic contents. The presence of derivatives of flavonoids has been found in many spices; moreover, numerous studies have conclusively shown that the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins, rather than from vitamins C, E and β -carotene [21, 37, 38]. Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress [21]. Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups [38]. It is also well known that phenolic compounds contribute to quality of food in terms of modifying colour, taste, aroma and flavour [39].

Prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered an important mode of anti-

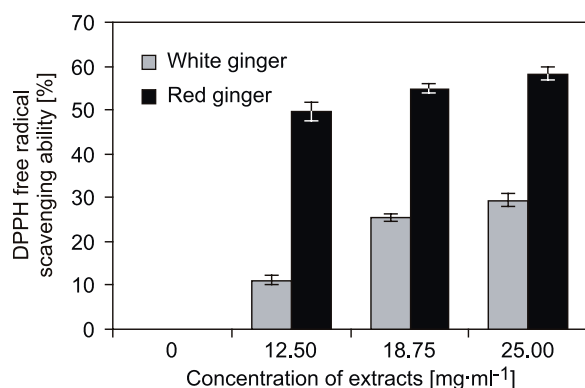


Fig. 3. DPPH free radical-scavenging ability of aqueous extract of ginger varieties.

Values represent mean \pm standard deviation, $n = 3$.

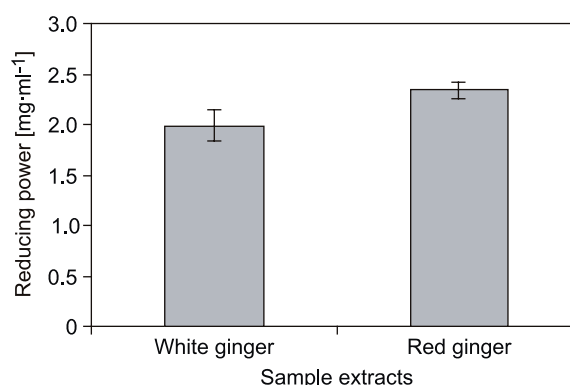


Fig. 4. Reducing power of aqueous extracts of ginger varieties.

Values represent mean \pm standard deviation, $n = 3$.

oxidant action [40]. DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule [41]. The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging [42]. The DPPH radical-scavenging ability of the ginger aqueous extracts (red and white) is presented in Fig. 3. The results revealed that both aqueous extracts scavenged DPPH radicals in a dose-dependent manner in the range of 0–25 mg·ml⁻¹. However, the aqueous extract of red ginger had a significantly higher ($P < 0.05$) free radical-scavenging ability than the aqueous extract of white ginger. This result followed the pattern observed with the phenolic contents (Tab. 1) and reducing activities (Fig. 4), where red ginger with higher phenolic content had significantly higher antioxidant activity. Earlier reports have established that antioxidant properties of plant food correlate with the phenolic contents [43, 44]. The antiradical activity of phenolics is principally based on the structural relationship between different parts of their chemical structures [45].

Reducing power is a novel antioxidation defence mechanism; the two mechanisms available to affect this property are by electron transfer and by hydrogen atom transfer [40]. This is because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of $\text{Fe}^{3+}/\text{Fe}^{2+}$, the values in the Ferric reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants [46]. The reducing powers of the aqueous extracts of red and white ginger were assessed based on their ability to reduce Fe^{3+} to Fe^{2+} and the results are presented in Fig. 4 as ascorbic acid equivalents. The results

revealed that the reducing power of red ginger extract was significantly higher ($P < 0.05$) than that of white ginger extract. This trend is in agreement with the total phenolic and flavonoid contents of the ginger varieties. Since the antioxidant activity of phenolics is mainly due to their redox activities, this allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [45].

CONCLUSION

Ginger is a good source of water-extractable phytochemicals with strong antioxidant activities and inhibitory activities against key enzymes linked to type-2 diabetes, namely, α -amylase and α -glucosidase. However, extract of white ginger with a lesser antioxidant activity possesses stronger anti-diabetic potential and thus offers a potential for use as dietary intervention in the management or control of postprandial hyperglycemia associated with type-2 diabetes.

REFERENCES

1. Ortiz-Andrade, R. R. – Garcia-Jimenez, S. – Castillo-Espana, P. – Ramirez-Avila, G. – Villalobos-Molina, R. – Estrada-Soto, S.: Alpha-glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: An anti-hyperglycemic agent. *Journal of Ethnopharmacology*, 109, 2007, pp. 48–53.
2. Shim, Y. J. – Doo, H. K. – Ahn, S. Y. – Kim, Y. S. – Seong, J. K. – Park, I. S. – Min, B. H.: Inhibitory effect of aqueous extract from the gall of *Rhuz chinensis* on alpha-glucosidase activity and postprandial blood glucose. *Journal of Ethnopharmacology*, 85, 2003, pp. 283–287.

3. Abesundara, K. J. – Matsui, T. – Matsumoto, K.: Alpha-glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, *Cassia auriculata*, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose. *Journal of Agricultural and Food Chemistry*, *52*, 2004, pp. 2541–2545.
4. Kwon, Y. I. – Jang, H. D. – Shetty, K.: Evaluation of *Rhodiola crenulata* and *Rhodiola rosea* for management of type II diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition*, *15*, 2006, pp. 425–432.
5. Kim, J. S. – Kwon, C. S. – Son, K. H.: Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Bioscience, Biotechnology and Biochemistry*, *64*, 2000, pp. 2458–2461.
6. Kim, Y. M. – Jeong, Y. K. – Wang, M. H. – Lee, W. Y. – Rhee, H. I.: Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition*, *21*, 2005, pp. 756–761.
7. Kotowaroo, M. I. – Mahomodally, M. F. – Gurib-Fakim, A. – Subratty, A. H.: Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects *in vitro*. *Phytotherapy Research*, *20*, 2006, pp. 228–231.
8. Ali, H. – Houghton, P. J. – Soumyanath, A.: Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, *107*, 2006, pp. 449–455.
9. Onal, S. – Timur, S. – Okutuku, B. – Zihnioglu, F.: Inhibition of alpha-glucosidase by aqueous extracts of some potent antidiabetic medicinal herbs. *Preparative Biochemistry and Biotechnology*, *35*, 2005, pp. 29–36.
10. Portnoi, G. – Chng, L. A. – Karimi-Tabesh, L. – Koren, G. – Tan, M. P. – Einarson, A.: Prospective comparative study of the safety and effectiveness of ginger for the treatment of nausea and vomiting in pregnancy. *American Journal of Obstetrics and Gynecology*, *189*, 2003, pp. 1374–1377.
11. Bliddal, H. – Rosetzky, A. – Schlichting, P. – Weidner, M. S. – Andersen, L. A. – Ibfelt, H. H. – Christensen, K. – Jensen, O. N. – Barslev, J.: A randomized, placebo-controlled, cross-over study of ginger extracts and ibuprofen in osteoarthritis. *Osteoarthritis Cartilage*, *8*, 2000, pp. 9–12.
12. Cady, R. K. – Schreiber, C. P. – Beach, M. E. – Hart, C. C.: GelstatMigraine (sublingually administered feverfew and ginger compound) for acute treatment of migraine when administered during the mild pain phase. *Medical Science Monitor*, *11*, 2005, pp. 165–169.
13. Penna, S. C. – Medeiros, M. V. – Aimbire, F. S. – Faria-Neto, H. C. – Sertie, J. A. – Lopes-Martins, R. A.: Anti-inflammatory effect of the hydralcoholic extract of *Zingiber officinale* rhizomes on rat paw and skin edema. *Phytomedicine*, *10*, 2003, pp. 381–385.
14. Bhandari, U. – Kanojia, R. – Pillai, K. K.: Effect of ethanolic extract of *Zingiber officinale* on dyslipidaemia in diabetic rats. *Journal of Ethnopharmacology*, *97*, 2005, pp. 227–230.
15. Chevallier, A.: The encyclopedia of medicinal plants. London : Dorling Kindersley, 1996. 336 pp. ISBN 9-780751-303148.
16. Ursell, A.: The complete guide to healing foods. London : Dorling Kindersley, 2000. 256 pp. ISBN 1-86466-291-3.
17. Srinivasan, K.: Plant foods in the management of diabetes mellitus: Spices as potential antidiabetic agents. *International Journal of Food Science and Nutrition*, *56*, 2005, pp. 399–414.
18. Sharma, M. – Shukla, S.: Hypoglycaemic effect of ginger. *Journal of Research in Indian Medicine, Yoga and Homeopathy*, *12*, 1977, pp. 127–130.
19. Mascolo, N. – Jain, R. – Jain, S. C. – Capasso, F.: Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *Journal of Ethnopharmacology*, *27*, 1989, pp. 129–140.
20. Akhani, S. P. – Vishwakarma, S. L. – Goyal, R. K.: Anti-diabetic activity of *Zingiber officinale* in streptozotocin-induced type I diabetic rats. *Journal of Pharmacy and Pharmacology*, *56*, 2004, pp. 101–105.
21. Oboh, G. – Puntel, R. L. – Rocha, J. B. T.: Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinense*, Habanero) prevents Fe²⁺-induced lipid peroxidation in brain - *in vitro*. *Food Chemistry*, *102*, 2007, pp. 178–185.
22. Bernfield, P.: Enzymes of starch degradation and synthesis. *Advances in Enzymology*, *12*, 1951, pp. 379.
23. Apostolidis, E. – Kwon, Y. I. – Shetty, K.: Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science and Emerging Technology*, *8*, 2007, pp. 46–54.
24. Singleton, V. L. – Orthofer, R. – Lamuela-Raventos, R. M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer, L. (Ed.): *Methods in enzymology*. Vol. 299 : Oxidants and antioxidants, Part A. San Diego, California : Academic Press, 1999, pp. 152–178.
25. Meda, A. – Lamien, C. E. – Romito, M. – Millogo, J. – Nacoulma, O. G.: Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chemistry*, *91*, 2005, pp. 571–577.
26. Gyamfi, M. A. – Yonamine, M. – Aniya, Y.: Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *General Pharmacology*, *32*, 1999, pp. 661–667.
27. Pulido, R. – Bravo, L. – Saura-Calixto, F.: Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *48*, 2000, pp. 396–3402.
28. Zar, J. H.: Biostatistical analysis. New Jersey : Prentice-Hall, 1984. 620 pp. ISBN 0-13-081542-X.
29. Zhang, J. – Kashket, S.: Inhibition of salivary amylase by black and green teas. *Caries Research*, *32*, 1998, pp. 233–238.
30. Nickavar, B. – Yousefian, N.: Inhibitory effects of six *Allium* species on α -amylase enzyme activity.

- Iranian Journal of Pharmaceutical Research, 8, 2009, pp. 53–57.
31. Kwon, Y. I. – Apostolidis, E. – Kim, Y. C. – Shetty, K.: Health benefits of traditional corn, beans and pumpkin: In vitro studies for hyperglycemia and hypertension management: Journal of Medicinal Food, 10, 2007, pp. 266–275.
 32. Bischoff, H.: Pharmacology of alpha-glucosidase inhibition. European Journal of Clinical Investigation, 24, 1994, pp. 3–10.
 33. Horii, S. – Fukase, K. – Matsua, T. – Kameda, K. – Asano, N. – Masui, Y.: Synthesis and α -D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potent oral antidiabetic agents. Journal of Medicinal Chemistry, 29, 1987, pp. 1038–1046.
 34. Kwon, Y. I. – Apostolidis, E. – Shetty, K.: In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresource Technology, 99, 2008, pp. 2981–2988.
 35. Li, W. G. – Zhang, X. Y. – Wu, Y. J. – Tian, X.: Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. Acta Pharmacologica Sinica, 22, 2001, pp. 1117–1120.
 36. Amic, D. – Davidovic-Amic, D. – Beslo, D. – Trinajstić, N.: Structure-radical scavenging activity relationship of flavonoids. Croatia Chemical Acta, 76, 2003, pp. 55–61.
 37. Marin, A. – Ferreres, F. – Tomas-Barberan, F. A. – Gil, M. J.: Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). Journal of Agricultural and Food Chemistry, 52, 2004, pp. 3861–3869.
 38. Materska, M. – Perucka, I.: Antioxidant activity of the main phenolic compounds isolated from hot pepper fruits (*Capsicum annuum* L.). Journal of Agricultural and Food Chemistry, 53, 2005, pp. 1730–1758.
 39. Memnune, S. – Ailal, Y. – Neva, G. – Bulent, C. – Zaynep, E. – Sezal, E.: Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pakistan Journal of Pharmaceutical Science, 22, 2009, pp. 102–106.
 40. Dastmalchi, K. – Dorman, H. J. D. – Kosar, M. – Hiltunen, R.: Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. Lebensmittel Wissenschaft und Technologie, 40, 2007, pp. 239–248.
 41. Je, J. Y. – Park, P. J. – Kim, E. K. – Ahn, C. B.: Antioxidant and angiotensin I converting enzyme inhibitory activity of *Bambusae caulis* in Liguamen. Food Chemistry, 113, 2009, pp. 932–935.
 42. Hu, C. – Zhang, Y. – Kitts, D. D.: Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys niger* var. *Henonis* leaf extract in vitro. Journal of Agricultural and Food Chemistry, 48, 2000, pp. 3170–3176.
 43. Chu, Y. – Sun, J. – Wu, X. – Liu, R. H.: Antioxidant and antiproliferative activity of common vegetables. Journal of Agricultural and Food Chemistry, 50, 2002, pp. 6910–6916.
 44. Ademiluyi, A. O. – Akpambang, V. O. E. – Oboh, G.: Polyphenol contents and antioxidant capacity of tropical clove bud (*Eugenia aromatica* Kuntze). Rivista Italiana delle Sostanze Grasse, 86, 2009, pp. 131–137.
 45. Rice-Evans, C. – Miller, N. J. – Paganga, G.: Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20, 1996, pp. 933–956.
 46. Halvorsen, B. L. – Holte, K. – Myhrstad, M. C. W. – Barikmo, I. – Hvattum, E. – Remberg, S. F. – Wold, A. B. – Haffner, K. – Bauge-rød, H. – Andersen, L. F. – Moskaug, J. O. – Jacobs, D. R. – Blomhoff, R. A.: Systematic screening of total antioxidants in dietary plants. Journal of Nutrition, 132, 2002, pp. 461–471.

Received 20 January 2010; revised 3 March 2010; accepted 8 March 2010.