Evaluation of the *in vitro* antioxidant potential of extracts obtained from *Cinnamomum zeylanicum* barks

Lidiya Georgieva, Dasha Mihaylova

Evaluation of the *in vitro* antioxidant potential of extracts obtained from *Cinnamomum zeylanicum*: *The present paper aimed to investigate extracts of the commonly used spice in the everyday life C. zeylanicum. The in vitro antioxidant activity of three different extracts was assessed by using DPPH and ABTS radical scavenging assays and reducing power method in addition to the total phenolic content evaluation. Positive correlation between results of all conducted analyses was established. The results obtained revealed the heat-reflux extraction as more effective compared to the traditional infusion method. However, the plant extracts of C. zeylanicum barks could be potential source of biological active substances.*

Key words: *Cinnamomum zeylanicum; phenolic content; antioxidant activity.*

INTRODUCTION

It is well known that bioactive compounds such as polyphenols are constituents of many plants and herbs, and they have attracted a great deal of public and scientific interest because of their health-promoting effects and of their potential applications in foods as antioxidants and antimicrobial agents. Nowadays scientists examined selected plants of interest in traditional diets in order to determine their phenolic composition, antioxidant capacity and antimicrobial activity.

Various plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore in recent years, considerable attention has been directed towards the identification of plants with antioxidant activity [5].

Dragland et al. [3] assessed the antioxidant capacity of 18 fresh herbs and 38 commercially available dried spices in Norway. They found oregano, sage, peppermint, and thyme to contain the greatest antioxidant capacity for fresh herbs, while cloves, allspice, and cinnamon contained the highest levels of antioxidant activity among dried spices. Clove, cinnamon, and oregano were found to posses the highest antioxidant capacity among the 26 spices tested by Shan et al. [11]. They also found a highly positive linear relationship between the total equivalent antioxidant capacity and total phenolic content (phenolic acids, phenolic diterpenes, flavonoids, and volatile oils). Dudonne et al. [4] reported that oak (*Quercus robur*), pine (*Pinus maritime*), and cinnamon (*Cinnamomum zeylanicum*) aqueous extracts possessed the highest antioxidant capacities in most of the methods used and could be potential sources of natural antioxidants.

Cinnamon (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, 10-15 meters tall, belonging to the family *Lauraceae*, native to Sri Lanka and South India. The flowers, which are arranged in panicles, have a greenish color and have a distinct odor. The fruit is a purple one-centimeter berry containing a single seed. Its flavor is due to an aromatic essential oil which makes up 0.5 to 1 % of its composition.

In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. The available *in vitro* and animal *in vivo* evidence suggests that cinnamon has antiinflammatory, antimicrobial, antibacterial, antioxidant, antitumor, cardiovascular, cholesterol-lowering, and immunomodulatory effects [2]. Aqueous extracts from cinnamon have been shown to increase *in vitro* glucose uptake and glycogen synthesis, increase phosphorylation of the insulin receptor and likely help trigger the insulin cascade system [6, 7].

Based on this the present study aimed to investigate the different possibilities for extracts obtaining of *C. zeylanicum* in order to optimize the everyday intake of biological active substances with potential health effects.

MATERIALS AND METHODS

Plant material

The cinnamon barks as dry samples were obtained from local pharmacies (Plovdiv, Bulgaria). After additional drying of the plant parts, they were roughly grounded and stored in air-tight dark containers until extraction.

Preparation of plant extracts

In order to investigate the biological potential of this plant species three different types of extracts were obtained. The procedures were conducted as follows:

- Water extract was obtained with boiled water for 30 min sucking until cooling. The final extract was filtered; the total volume was filled up to 30 ml with water and stored at 4 °C without adding any preservatives until use.
- Ethanol and methanol extracts were prepared using conventional heat reflux extraction. 0.5 g grounded plant material was subjected to heat-reflux extraction with 30 ml of the appropriate solvent for 30 minutes. The final extracts were filtered, the total volume was filled up to 30 ml with the solvent applied and were stored at 4 °C without adding any preservatives until use.

Determination of Total Phenolic Content (TPC)

The total phenolic content in the different C. *zeylanicum* extracts was determined spectrophotometrically using the Folin–Ciocalteu phenol reagent [8]. The TPC in the extracts was expressed in terms of gallic acid equivalent (mg GAE)/g leaf dry weight (DW).

Antioxidant activity assays

The antioxidant activity of the plant extracts was determined by *in vitro* methods: DPPH and ABTS radical scavenging activity assays and reducing power method. All assays were carried out in triplicate and the average values were considered and expressed as mean ± SD.

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined [1] after 15 min incubation at room temperature and the light absorption was measured at 517 nm. The results were expressed as function of the concentration of Trolox (TEAC, mM TE/g DW).

ABTS radical cation decolorization assay

The radicals scavenging activity of the extracts against radical cation ($ABTS^{\bullet+}$) was estimated according to a previously reported procedure with some modifications [910]. The results were expressed as TEAC value (mM TE/g DW).

Reducing Power

The reducing power of the extracts was determined according to the method of Oyaizu [9]. Vitamin C was used as positive control.

RESULTS AND DISCUSSION

Total polyphenolic content

Since polyphenols significantly contribute to the overall antioxidant activity, it was reasonable to determine their total amount in the investigated extracts. The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent ranged from 11.39 ± 0.78 to 57.7 ± 1.32 mg GAE/g DW (Table 1.) and the highest concentration was established in the methanol extract.

Polyphenolic compounds are some of the most effective antioxidative constituents in plant foods such as fruits, vegetables, and grains; thus it is important to quantify their polyphenolic contents and to assess their contribution to antioxidant activity. Therefore as next step in the present research the antioxidant capacity of the obtained extracts was evaluated.

Table 1. Total phenol content and antioxidant activity of extracts obtained from *C. zeylanicum*

Extract /Assay	TPC, mg GAE/g DW	$TEAC_{DPPH}$, mM TE/g DW	$TEAC_{ABTS'}$ mM TE/g DW
Water extract	11.39 ± 0.78	4.98 ± 0.09	0.47 ± 0.01
Ethanol extract	51.43 ± 2.94	16.94 ± 1.15	1.28 ± 0.03
Methanol extract	57.7 ± 1.32	18.16 ± 1.34	1.92 ± 0.02

Antioxidant activity (AOA)

In order to investigate the AOA of the obtained plant extracts, experiments with two stable radicals - $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ were conducted. In addition to the reducing power assay was performed.

The antioxidant capacities of different bark extracts of *C. zeylanicum* varied in wide range from 0.47 ± 0.01 to 18.16 ± 1.34 mM TE/g DW (Table 1.). $TEAC_{DPPH}$ values were in range from 4.98 ± 0.09 to 18.16 ± 1.34 mM TE/g DW and the $TEAC_{ABTS}$ values were from 0.47 ± 0.01 to 1.92 ± 0.02 mM TE/g DW, respectively. Higher TEAC value indicates that a sample has stronger AOA. These results were in agreement with the established total phenol content (Table 1.).

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. High absorbance indicates high reducing power (Fig. 1.). The methanol and ethanol extracts had higher reducing power than the water one. The reducing power of the C. *zeylanicum* methanol extract was 0.668, and it was comparable with that of the vitamin C, 0.08 mg/ml. The reducing power of the 96 % ethanol extract was 0.412, followed by water extract with lower values. For the purpose of the assay all samples were diluted 20 fold.

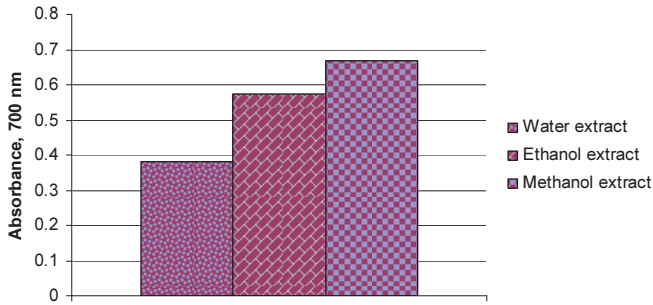

Fig. 1 Reducing power of different extracts of C. *zeylanicum* barks

In brief, among the investigated extract procedures the highest results were established by the methanolic extract. The observed differences could be explained by the different polarity of the polyphenol compounds present in the investigated plant. However the water as solvent is not so effective in terms of obtaining of phenolic substances with antioxidant potential despite it is the most widely used solvent in household. The

established variations between the results could be due to the basic principle of the extraction procedures applied as well.

In addition, a quantitative analysis of the relationship between the established antioxidant activities and the present in the different extracts phenolic substances was also performed.

Table 2. Correlation coefficients (r) – relationship between the conducted analyses

	$TEAC_{DPPH}$	$TEAC_{ABTS}$	Reducing power
TPC	0.9991	0.9457	0.9784
$TEAC_{DPPH}$		0.9315	0.9690
$TEAC_{ABTS}$			0.9925

Table 2 shows the correlation coefficients (r) between the two conducted analyzes on the antioxidant activity and the content of phenolic substances. As shown, a significant positive correlation (r is from 0.9315 to 0.9991) was observed between the total phenolic content and TEAC values, which indicates significant contribution of phenolic substances to the established antioxidant activity. Reported positive correlations indicate that the antioxidant activity of the tested *C. zeylanicum* bark extracts may due to the presence of phenolic compounds. The identification in particular of these substances should be a subject of detailed analysis in a further research.

CONCLUSIONS

In the present study the antioxidant properties and the total phenolic content of different extracts obtained from *C. zeylanicum* were investigated. Among the applied extraction solvents the heat-reflux extraction with methanol shows the best results in terms of the highest antioxidant activity and polyphenolics concentration. The established positive correlation between the concentration of the phenolic substances in the samples and the demonstrated antioxidant activity, suggest their important role.

The results obtained revealed the possibility of using the test plant as natural sources of antioxidants, which may be beneficial for the human health.

REFERENCES

[1] Brand-Williams,W., M.E.Cuvelier, C.Berset, Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie - Food Science & Technology, 1995, 28, 25-30.

[2] Charles Denys, J. Antioxidants, Antioxidant Properties of Spices, Herbs and Other Sources. Springer Science & Business Media, 2012.

[3] Dragland, S., H. Senoo, K. Wake, K. Holte, R. Blomhoff. Several culinary and medicinal herbs are important sources of dietary antioxidants. Journal of Nutrition, 2003, 133(5), 1286–1290.

[4] Dudonne, S., X. Vitrac, P. Coutiere, M. Woillez, J.M. Merillon. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays. Journal of Agricultural and Food Chemistry, 2009, 57, 1768-1774.

[5] El-Hela, A., A. Abudullah. Antioxidant and antimicrobial activities of methanol extracts of some Verbena species: *In vitro* evaluation of antioxidant and antimicrobial activity in relation to polyphenolic content. Journal of Applied Sciences Research. 2010, 6, 683-689.

[6] Imparl-Radosevich, J., S. Deas, M.M. Polansky, D.A. Baedke, T.S. Ingebritsen, R.A. Anderson, D. Graves. Regulation of PTP- I and insulin receptor kinase by fractions from cinnamon: implications for cinnamon regulation of insulin signalling. Hormone Research, 1998, 50, 177-182.

[7] Jarvill-Taylor, KJ, R.A. Anderson, D. Graves. A hydroxychalcone derived from cinnamon functions as a mimetic in 3T3-LI adipocytes. Journal of American Collegen of Nutrition, 2001, 20, 327-336.

[8] Kujala,T.S., J.M. Loponen, K.D. Klika, K. Pihlaja, Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds, Journal of Agricultural and Food Chemistry, 2000, 48, 5388-5342.

[9] Oyaizu, M. Studies of products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. Jpn. J.Nutr. 1986, 44, 307–315.

[10] Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C.A. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 1999, 26, 1231-1237.

[11] Shan, B., Y.Z. Cai, M. Sun, H. Corke. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry, 2005, 53, 7749-7759.

About the authors:

Lidiya Georgieva, PhD student, Department of Organic Chemistry, University of Food Technologies, 26 Maritza Blvd., Plovdiv, Bulgaria, e-mail: lid_georgieva@abv.bg

Dasha Mihaylova, PhD, Department of Biotechnology, University of Food Technologies, 26 Maritza Blvd., Plovdiv, Bulgaria, e-mail: dashamihaylova@yahoo.com

This paper has been reviewed