ISSN 2449-8955

European Journal of Biological Research

Research Article

# Antioxidant profile of essential oils and extracts of cinnamon bark (*Cinnamomum cassia*)

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**Received:** 06 October 2016; **Revised submission:** 05 December 2016; **Accepted:** 08 December 2016



**DOI**: http://dx.doi.org/10.5281/zenodo.197200

## ABSTRACT

Nowadays, more and more interest is focused on compounds that inhibit reactions promoted by free radicals. The study shows the antioxidant activity of essential oils isolated from *Cinnamomum cassia*. The methanolic extracts were also obtained and its scavenging ability investigated. The $IC_{50}$ value, defined as the concentration of the sample required for 50% scavenging of DPPH free radicals, was also estimated. DPPH and ABTS assay indicated that lower $IC_{50}$ value for cinnamon extracts corresponds to its higher radical scavenging activity ($IC_{50}$ = 42.03 µg/L and $IC_{50}$ = 5.13 µg/L, respectively). Thus, extracts are considered to be better radical scavengers than essential oils ($IC_{50\ DPPH}$ = 147.23 µg/L and $IC_{50\ ABTS}$ = 64.51 µg/L).

**Keywords:** Cinnamon; Antioxidant activity; DPPH; ABTS; Essential oil; Extracts.

## 1. INTRODUCTION

In ancient times, cinnamon (from the Greek *kinamon*), also known as a sweet wood, was greatly treasured by Egyptians, Israelites and Phoenicians. It was commonly used as a body perfume and medicine, and proclaimed as one of the main ingredients in the love potions prepared by the Greek and Romans as well. Several varieties of cinnamon can be distinguished, but only two types are generally used: Cassia (Chinese cinnamon) and Canela (Ceylon cinnamon) [1].

*Cinnamomum cassia* (Lauraceace) belongs to an evergreen tree originating in southern China, and widely cultivated in the countries of southern and eastern Asia (India, Malaysia, Thailand, Vietnam, Indonesia, and Laos) [2]. Cassia is characterized by a sweet spicy aroma, more pungent bitter taste and thicker bark than Ceylon cinnamon [3]. The dried barks of Cassia are not only known as a spice and flavoring agent, but also are regarded as a traditional medicine in the world [4]. It is commonly used for the treatment of amenorrhea, rheumatoid arthritis, cardiac palpitation, diarrhea, and gastrointestinal neurosis [4, 5]. Pharmacological studies have demonstrated that Cassia cinnamon possesses antidiabetic [6, 7], antioxidant [5, 8], antibacterial [9], antifungal [10], antiulcer [11], antihyperlipidemic [12], and analgesic activity [2, 13]. Recently, an increasing number of investigations on antioxidant activity of spices have been reported [14-17].

This study reports the antioxidant activity of the extracts and essential oils from *Cinnamomum*

*cassia*. The chemical composition of cinnamon oil was also identified.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and instrumentation

2-2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (≥99.0%) and (±)-6-hydroxy-2,5, 7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich. Methanol (99.8%) and Hexane (99.5% n-hexane) were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland).

GC-MS analyses were carried out using a Trace GC Ultra gas chromatograph connected with a DSQ II mass spectrometer (Thermo Electron Corp., Waltham, Ma., U.S.A.). Chromatographic separations were performed on Rtx-1 nonpolar capillary column (30 m × 0.32 mm; 0.25 μm film thickness). Preparations of extracts were carried out into an ultrasonic bath (InterSonic S.C., Olsztyn, Poland) and separator Labofuge 300, Thermo-Scientific (Waltham, MA, USA). Antioxidant measurements were taken on a Hewlett-Packard 8453 spectrophotometer, (Waldbronn, Germany).

### 2.2. Plant material

Sticks of cinnamon (*Cinnamomum cassia*) bark were purchased from a local store in Lodz, Poland. Spice was imported from China.

### 2.3. Isolation of essential oil

Sticks of cinnamon bark (*C. cassia*) (40.0 g) were immersed in 600.0 mL water in a round-bottom flask. The essential oil was obtained by 3-hours-continuous hydrodistillation of cinnamon in an apparatus - the modification of Deryng instrument for analytical isolation of essential oil by hydrodistillation - constructed in the Institute of General Food Chemistry [18]. This apparatus in particular does not release any odors and separates phases very well. The isolation of essential oil was carried out in triplicate, and the results were expressed as mean values ± standard deviation (SD).

### 2.4. Gas chromatography-mass spectrometry (GC-MS) of essential oil

Essential oil was analyzed according to a procedure described by Brodowska et al. [15]. The temperature program for Rtx-1: 60 to 300°C at 4°C/min. The injector (SSL) temperature was 280°C, and transfer line temperature 200°C. Helium was used as the carrier gas, flow rate 1 mL/min, split ratio 1:20. The identification of the essential oil components was based on a comparison of their retention indexes (RI), mass spectra (NIST and Wiley libraries), and literature data [19, 20].

### 2.5. Preparation of extracts

Cinnamon bark extract was obtained by triple extraction of 0.5 g of the material, which was ground before, with 4.0 mL 70% methanol. Then, sample was mixed by vortex (1 minute) and placed into an ultrasonic bath (10 minutes). The mixture was centrifuged at 2500 rpm for 5 minutes at room temperature. The supernatant was decanted (10 mL volumetric flask) and the next portion of extractant (3.0 mL 70% methanol) was added to residue. The above steps were repeated twice. All three supernatants were mixed and diluted till the mark (10 mL). The obtained extract (10 mL each) was recovered and stored in the refrigerator until analysis.

### 2.6. Antioxidant activity

#### *2.6.1. DPPH radical scavenging activity*

The total antioxidant capacity of the methanolic extracts as well as the essential oils was determined spectrophotometrically, following the modified procedure described by Hatano et al. [21] taking into account the method of Brand-Williams et al. [22]. The absorbance was measured 30 minutes after the initial mixing of different concentrations of the cinnamon extracts and oils (final concentration 5-50 μg/L) with 1.95 mL of DPPH solution at 515 nm, and quantified using Trolox as a standard. The results were expressed as mg Trolox equivalent TE/g of a sample. Inhibition of DPPH radical was measured as the decrease in absorbance of the samples versus DPPH standard

solution. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The antiradical activity was expressed as $IC_{50}$ (μg/L), which is defined as the extract concentration necessary to scavenge 50% DPPH free radicals. The determination was carried out in triplicate, and the results were expressed as mean values ± standard deviation (SD).

#### *2.6.2. $ABTS^+$ radical scavenging activity*

$ABTS^+$ assays were done in accordance with the modified method of Re et al. [23]. ABTS radical cation ($ABTS^+$) was generated through the reaction between ABTS stock solution and potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Such obtained the $ABTS^+$ was diluted with methanol to an absorbance 0.70 (±0.02) at 734 nm. Diluted $ABTS^+$ solution ($A_{734nm}$=0.70 (±0.02)) was used to measure the reduction of the radical cation caused by essential oil and extract, determined by the decolorization of the $ABTS^+$. The absorbance was measured 5 minutes after the initial mixing of different concentrations of the cinnamon extracts and oils (final concentration 5–50 μg/L) with 1 mL of $ABTS^{+\bullet}$ solution at 734 nm. Three independent experiments were carried out for each of the samples, and the results were expressed as Trolox equivalent antioxidant capacity.

### 2.7. Statistical analysis

All determinations were carried out in triplicate. Mean values with standard deviations (±SD) were reported for each case. Statistical analysis (means, standard deviation) and analysis of variance (One-Way ANOVA) were conducted using OriginPro 8., Microcal, Northampton, MA, USA, 2007.

## 3. RESULTS AND DISCUSSION

### 3.1. Essential oil characterization

The composition of the essential oil (EO) of cinnamon bark (*Cinnamomum cassia*) is shown in Table 1. The essential oil profile from cinnamon bark contains more than 30 compounds, out of which 20 were identified, contributing 95.89% of the total essential oil content of bark. The results were compared with the retention indices (RIs) of authentic samples and their mass spectra with those of standard libraries (NIST and Wiley) and the literature [19, 20].

The main compound of cinnamon EO is (*E*)-cinnamaldehyde, which is considered as the most dominant one. The major monoterpene in cinnamon oil was β-pinene (1.08%). Other monoterpenes presented in a lower percentage were sabinene (0.72%), linalool (0.94%), and camphor (0.86%). The compounds in a moderate percentage were assigned to benzaldehyde (2.22%), bornyl-acetate (1.06%), and (*E*)-cinnamyl acetate (1.48%). The area percentages of other volatile compounds were primarily less than 1%. According to Raghavan [1] essential oil of cinnamon contains mainly cinnamaldehyde (65%-95%), cinnamyl acetate, cinnamic acid, benzaldehyde, small amounts of coumarin, and trace amounts of eugenol, which was confirmed in our studies.

### 3.2. Antioxidant activity

Natural antioxidants, presented as essential oils, are characterized by a wide mechanism of action. Therefore, a single method of antioxidant activity is unable of comprehending the antioxidant profile, thus different assays of antioxidant activity should be used [5, 15]. Accordingly, in the present study the methanolic extracts and essential oils were examined for their free radical scavenging capacity towards the DPPH and ABTS method, which present different mechanisms of the determination of antioxidant capacity [23, 24].

The inhibitory effects of essential oils and extracts on the DPPH radical are given in Table 2. The average scavenging ratio for DPPH increases with the increasing concentrations of the samples. The TEAC (Trolox Equivalent Antioxidant Capacity) value recorded for DPPH presents the highest scavenging power for the highest concentration tested (50 μg/L). Significant scavenging capacity is observed for cinnamon extracts (255.20 mg/L). Furthermore, cinnamon extracts are characterized by a steady increase in the DPPH radical scavenging capacity among the lowest and highest concentration (237.47-255.20 mg/L).

**Table 1.** GC-MS analysis of the composition of essential oil of cinnamon bark (*Cinnamomum cassia*).

| No | Compounds | RI[a] | % |
|---|---|---|---|
| **1** | Benzaldehyde | 927 | 2.22±0.29 |
| **2** | β-Pinene | 929 | 1.08±0.04 |
| **3** | Camphene | 941 | 0.59±0.03 |
| **4** | Sabinene | 968 | 0.72±0.03 |
| **5** | Linalool | 1085 | 0.94±0.09 |
| **6** | (*E*)-Cinnamyl alcohol | 1125 | 0.37±0.03 |
| **7** | Camphor | 1142 | 0.86±0.06 |
| **8** | Borneol | 1159 | 0.17±0.01 |
| **9** | Benzenepropanal | 1162 | 0.62±0.07 |
| **10** | α-Terpineol | 1172 | 0.40±0.01 |
| **11** | (*Z*)-Cinnamaldehyde | 1182 | 0.43±0.05 |
| **12** | Decanal | 1201 | 0.13±0.03 |
| **13** | (*E*)-Cinnamaldehyde | 1239 | 82.85±1.36 |
| **14** | Bornyl acetate | 1269 | 1.06±0.08 |
| **15** | Eugenol | 1354 | 0.13±0.02 |
| **16** | Geranyl acetate | 1390 | 0.29±0.02 |
| **17** | (*E*)-Cinnamyl acetate | 1407 | 1.48±0.65 |
| **18** | Caryophyllene | 1416 | 0.32±0.03 |
| **19** | Ethyl cinnamate | 1422 | 0.46±0.05 |
| **20** | α-Calacorene | 1589 | 0.77±0.09 |
| | **Total** | | **95.89±3.40** |

Notes: [a]Retention index (RI) is an average of all RIs in analysed samples. The results obtained were expressed as mean ± SD with n = 3 according to One-Way ANOVA.

**Table 2.** The trolox equivalent antioxidant capacities of cinnamon extracts and essential oils calculated with respect to the DPPH and ABTS assays.

| Sample | Concentration (µg/L) | $TEAC_{DPPH}$ (mg/L) | $IC_{50}$ (µg/L) | $TEAC_{ABTS}$ (mg/L) | $IC_{50}$ (µg/L) |
|---|---|---|---|---|---|
| **Cinnamon extract** | 5.0 | 237.47 ± 0.14 | 42.03 ± 0.06 | 251.22 ± 0.07 | 5.13 ± 0.07 |
| | 8.3 | 247.69 ± 0.86 | | 648.67 ± 0.96 | |
| | 12.5 | 252.88 ± 0.06 | | 892.36 ± 0.74 | |
| | 25.0 | 254.07 ± 0.27 | | 2493.09 ± 0.62 | |
| | 50.0 | 255.20 ± 0.02 | | 6979.58 ± 0.92 | |
| **Essential oils of cinnamon** | 5.0 | 94.96 ± 0.70 | 147.23 ± 0.04 | 238.87 ± 0.32 | 64.51 ± 0.09 |
| | 8.3 | 100.12 ± 2.39 | | 462.13 ± 0.63 | |
| | 12.5 | 120.58 ± 6.23 | | 756.55 ± 0.39 | |
| | 25.0 | 236.32 ± 1.47 | | 903.46 ± 0.78 | |
| | 50.0 | 242.54 ± 2.24 | | 1134.08 ± 0.91 | |

In contrast, cinnamon oil possess a relatively high increase in antioxidant activity with further increase in concentration (94.96-242.54 mg/L). The $IC_{50}$ value, defined as the concentration of the sample required for 50% scavenging of DPPH free radicals, was also calculated. A smaller $IC_{50}$ value corresponds to a greater potency [25]. The $IC_{50}$ values (Table 2) indicate that extracts (42.03 µg/L) are more effective DPPH radical inhibitor than essential oil of cinnamon bark (147.23 µg/L). Udayaprakashet al. [26] evaluated that $IC_{50}$ values for methanolic extract of cinnamon were significantly higher than $IC_{50}$ values in this study. Our results are also in disagreement with those reported by Bozan et al. [27], whereas extract of *C. cassia* showed higher scavenging activity.

The total antioxidant capacities ($TEAC_{ABTS}$) of the tested samples (Table 2) present a wide variation. TEAC values are the highest for the highest concentration (50 µg/L) among all samples. The highest antioxidant capacity is assigned to cinnamon extracts (6979.58 mg/L). The $IC_{50}$ value calculated for $TEAC_{ABTS}$ (Table 2) shows that the ABTS radical cation inhibitor is much more effective for extract of *C. cassia* (5.13 µg/L) than its essential oil (64.51 µg/L). According to Mathew and Abraham [28], the TEAC value for cinnamon extracts at the maximum concentration studied were found to be 18.45 µg/mL, which suggests higher scavenging activity. Udayaprakash et al. [26] also reported a good ability to scavenge ABTS radical cation (99.36%).

The relatively small changes of cinnamon extracts and oils in antioxidant activity for DPPH assay may be caused by several factors. Various relative scavenging capacity of cinnamon samples against different testing radicals can be explained by the different mechanisms involved in the radical-antioxidant reactions. The stoichiometry of the $ABTS^{+}$ and $DPPH^{\bullet}$ reactions between the anti-oxidant compounds in the extracts and oils is quite different, thus this could be one of the main reasons for the difference in their scavenging potential. Furthermore, the one electron reduction potential of the $DPPH^{\bullet}$ and $ABTS^{+}$ with respect to the compounds in the tested samples are different which can also have influence on their difference in response time. Stereoselectivity of the radicals or the solubility of the extracts and oils in different testing systems are also considered to be important factors to affect the capacity of the cinnamon extracts and oils to react and quench different radicals [28, 29].

## 4. CONCLUSIONS

The results obtained by the use of two different methods (DPPH and ABTS) proved that cinnamon bark, its methanolic extracts and essential oils can be considered as a good source of natural antioxidants. High correlation between antioxidant activity and cinnamon oil and extract may be attributed either to high concentration of the bioactive constituents or to synergy among different oil and extract constituents. Both extract and essential oil of cinnamon bark could be applied in cosmetic and pharmaceutical branches of industry.

## ACKNOWLEDGMENTS

This work was financially support by Statute Funds No. I28/DzS/9184.

## AUTHORS' CONTRIBUTIONS

KMB conceived and designed research. AJB and KMB conducted experiments (AJB - Isolation of essential oil and its characterization; KMB - Preparation of extracts, Determination of its Antioxidant activity and Statistical analysis). KŚ provided the plant material and shared Deryng apparatus for isolation of essential oil. EŁCh analysed data and references, as well as took care of the adjustment of the language. All authors read and approved the final manuscript.

## TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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