

Determination of Total Phenolic Content, Radical Scavenging Activity and Total Antioxidant Capacity of Cinnamon Bark, Black Cumin Seeds and Garlic

Wijewardhana U.S.¹, Gunathilaka U.G.S.A.², Navaratne S.B.³

¹Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka-10250

Abstract— Spices and herbs are enriched with natural antioxidant compounds which impart a higher antioxidant vigor for those products. Garlic, cinnamon and black cumin seeds are commonly used spices and their total phenolic content, radical scavenging activity and total antioxidant activity were evaluated. Cinnamon shows the highest total phenolic content (TPC) yielding 18.94 ± 0.46 GAE mg/100g of the dry weight. It was cited highest in total antioxidant capacity (TAC) and DPPH radical scavenging activity, yielding 149.15 ± 1.73 mg/ GAE and IC_{50} value of 0.009 ± 0.76 respectively. Garlic showed the lowest TPC 5.46 ± 0.26 GAE mg/100g and yielded IC_{50} value of 2.446 ± 0.34 and 5.136 ± 0.636 mg GAE/g for TAC assay. Black cumin seeds cited the highest IC_{50} value (2.935 ± 0.02) and lowest total antioxidant capacity (4.887 ± 0.044 mg GAE/g) while yielding a moderate value for TPC (8.45 ± 1.81 mg GAE/100g of dry weight).

Keywords— Total phenolic content: Free radical scavenging activity: Total antioxidant capacity: Cinnamon: Garlic: Black cumin.

I. INTRODUCTION

An antioxidant is any constituent that, significantly delays or prevents the oxidization of the substrate when present it in a material¹, are two types such as synthetic antioxidants and natural antioxidants. Natural antioxidants are commonly derived from plant sources. Cinnamon bark, black cumin seeds and garlic are commonly used spices all over the world which are rich in antioxidant compounds^{2,3,4}. Since ancient times, these spices have been used as an ingredient and for seasoning in many food stuffs and also in medicinal purposes⁵. Garlic (*Allium sativum*) is believed to have the ability to prevent and treat heart and metabolic diseases, such as atherosclerosis, thrombosis, hypertension, dementia, cancer, and diabetes^{6,7,8}. Garlic possesses antioxidant and free radical scavenging activities at low concentrations, containing two main classes of antioxidant compounds; flavonoids (flavones and quercetins) and sulfur-containing compounds (allyl-cysteine, diallyl sulfide, and allyl trisulfide)⁶. Cinnamon (*Cinnamomum zeylanicum*) is an evergreen tree which is native to Sri Lanka, has a reputation as a cure for cold and has been used to treat diarrhoea and other stomach problems. Research studies suggest that cinnamon has anti-inflammatory, antimicrobial, antibacterial, antioxidant, antitumor and cardiovascular cholesterol lowering properties⁹. Further, Cinnamon contains a number of antioxidant components including vanillic, caffeic, gallic, protochatechuic, p-hydroxybenzoic, p-coumaric, and ferulic acids and p-hydroxybenzaldehyde⁶. Black seeds/ black cumin (*Nigella sativa*) is traditionally used as a form of medicine and

nutrition. It has been used to treat fever, headache, anxiety, diarrhea, asthma, and stroke, and is known to be highly anti-inflammatory. Black cumin seeds are rich in phenolic compounds and therefore it has a potential as an antioxidant agent¹⁰. Much of the biological activity of *N. sativa* has been shown to be due to thermoquinone, which is now considered the active component of its essential oil^{4,11}. Since extracts of these spices have a potential to be used as natural antioxidants instead of synthetic antioxidants, the study was done for evaluating total phenolic content, DPPH radical scavenging activity and the total antioxidant capacity of cinnamon bark, garlic and black cumin seeds.

II. MATERIALS AND METHODOLOGY

A. Material Collection

Cinnamon bark (*Cinnamomum zeylanicum*) samples were collected from Cinnamon Research Station in Peradeniya, Sri Lanka. Garlic (*Allium sativum*) and Black cumin (*Nigella sativa*) seeds were collected from registered Ayurvedic-herbal material supplier in Sri Lanka. All chemicals used for chemical testing were of analytical grade with highest purity available (>99.5%).

B. Preparation of the Extract

Garlic samples were peeled off, disintegrated into small pieces and dried in a hot air oven (60°C) for six hours. Cinnamon samples were disintegrated to small pieces and dried at 60°C for one hour to remove the free water present in it. Black cumin seeds were also dried in the same manner. Dried samples were ground using a heavy grinder to result a fine powder. 4g of powder was taken and extracted to methanol 40 ml (1: 10 ratio) using the ultrasonic processor (Chromtech) for 20 minutes. Methanolic fraction was separated by centrifuging at 6000 rpm for 10 minutes. Resulted methanolic fraction was evaporated using a vacuum dryer under low pressure. A known weight of obtained extract was dissolved with a known volume of methanol to have an extract with a known concentration. Obtained extract was stored at 4°C until analysis.

C. Determination of Total Phenolic Content

Total phenolic content of cinnamon, garlic and black cumin seeds were determined using the Folin-Ciocalteu reagent according the method described by Singleton and Rossi (1965)¹² with modifications. An aliquot of 0.5 ml of extracts (all extracts were diluted to have the same

concentration of 5 mg/ml) were added to 2.5 ml of distilled water followed by the addition of F-C reagent 0.5 ml. The mixture was incubated at room temperature for 6 minutes. Then, 5 ml of 7% sodium carbonate was added. To that mixture, 2.5 ml of distilled water was added and incubated under room temperature for 90 minutes. The absorbances of the extracts were measured at 760 nm against a blank. Total phenolic content of cinnamon, garlic and black cumin seeds was expressed as Gallic acid equivalents (GAE) per gram of dry weight using the calibration curve of Gallic acid. Standard curve was constructed using the same methodology. The stock solution was made by dissolving 1g of Gallic acid anhydrous in 100 ml of methanol (corresponding to the concentration of 10 mg/ml). Dilution series was prepared using the stock solution in a range covering 10 mg/ml to 0.1 mg/ml.

D. Determination of Free Radical Scavenging Activity

DPPH radical scavenging activity was determined according to the method described by Brand-Williams, Cuvelier and Berset, (1995)¹³ with modifications. DPPH stock solution was prepared by dissolving 0.0032g of DPPH in 100ml of methanol. The prepared solution was incubated for 30 minutes at room temperature in a dark place to obtain an initial absorbance of 0.900±0.02 at 517 nm. Dilution series consisting of six suitable concentrations of the plant extracts was prepared by using methanol as the solvent. 0.5 ml of the diluted extracts were added in triplicates to stopper tubes and for each tube 2.5 ml of DPPH solution was added. A control was prepared by adding 2.5 ml of DPPH to 0.5 ml of methanol. Thereafter, solutions were vortexed to mix well and incubated again in a dark condition for 30 minutes. After 30 minutes, absorbance was measured against the blank. Free radical inhibition percentage was calculated using the following equation.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}} \times 100$$

The calculated inhibition percentage of absorbance at 517nm was plotted as a function of concentration of samples. The sample concentration which gives the 50% inhibition activity was estimated as the IC₅₀ value from regression analysis using the MINITAB 17 software. Calibration curve was prepared using the Gallic acid as the standard following the same methodology.

E. Determination of Total Antioxidant Capacity

Total antioxidant capacity of cinnamon, garlic and black cumin seeds was determined using the Phosphomolybdenum reagent according to the method described by Prieto, Pineda and Aguilar, (1999)¹⁴ with modifications. Initially, 1mg/ml solutions were prepared from all antioxidant sources by diluting with methanol. From each solution, 0.3ml aliquot was added to test tubes in triplicates. For that, 3 ml of phosphomolybdenum reagent was added, test tubes are covered, vortexed and incubated for 90 minutes at 95°C. Finally, absorbance was measured using the UV-Visible spectrophotometer at 695 nm against a blank.

Standard curve was constructed using the same methodology as described above. The stock solution was made by dissolving 1g of Gallic acid anhydrous in 100 ml of methanol (corresponding to the concentration of 10 mg/ml). Dilution series was prepared using the same stock solution in a range covering 10 mg/ml to 0.1 mg/ml.

F. Statistical Analysis

All tests were carried out in triplicates. Obtained data was analysed using Minitab 17 statistical software and differences were considered statistically significant when p<0.05.

III. RESULTS AND DISCUSSION

TABLE I. TPC, Radical scavenging activity and TAC of garlic, cinnamon bark and black cumin seeds

Sample	Total phenolic content (mg GAE/100g)	DPPH radical scavenging activity based on IC ₅₀ value (mg/ml)	Total antioxidant capacity (mg GAE/g)
Garlic	5.46 ± 0.26 ^c	2.446±0.34	4.887 ± 0.044 ^b
Cinnamon	18.94 ± 0.46 ^a	0.009±0.76	149.15 ± 1.73 ^a
Black cumin	8.45 ± 1.81 ^b	2.935±0.02	5.136 ± 0.636 ^b

A. Determination of Total Phenolic Content

Total phenolic contents of garlic, cinnamon bark and black cumin seeds were calculated as Gallic acid equivalents, mg per 100g of the dry weight and results are illustrated in table 1.

Phenolic compounds are secondary plant metabolites bearing an aromatic ring and having one or more hydroxyl substituents. Total phenolic content was measured by using Folin-Ciocalteu reagent as Gallic acid equivalents. According to the results given in the table 1, total phenolic content of the cinnamon bark extract is highest among the sources, yielding 18.94 mg GAE per 100g of the dry weight. The result obtained by ⁹, was 57.7 ± 1.32 mg GAE/g for the dry powder of the Cinnamon bark. And according to ¹⁵, cinnamon was cited with TPC of approximately 15 mg GAE/g of the sample which is very close to the value obtained by the study. According to the results, garlic cited the lowest phenolic content yielding 5.46± 0.26 mg Gallic acid equivalents per 100g of the dry weight. As per the results obtained by ¹⁶, TPC of raw garlic was 11.21 ±2.65 mg GAE per 100g for dry weight. From the obtained results, black cumin shows a moderate value for total phenolic content having 8.45 ± 1.81 mg GAE/ 100g of the dry weight. According to the results obtained by ¹⁷ total phenolic content of turkey variety is 2.92 mg GAE/g of the dry extract. The results obtained from the study were compatible with previous research however, variation of values might be due to differences of origin of the raw materials under different geoclimatologically variations.

B. Determination of Free Radical Scavenging Activity

Methanolic extracts of garlic, black cumin seeds and cinnamon bark were evaluated by DPPH test, in order to investigate their radical scavenging activity. Results of IC₅₀ value of them are illustrated in the table 1.

DPPH is a stable radical widely used to evaluate the free radical scavenging activity in many plant extracts. DPPH shows a maximum absorbance at 515-517 nm and undergo

reduction by antioxidants. The disappearance of DPPH in the presence of antioxidants is measured spectrophotometrically at 517 nm. From the absorbance obtained, IC₅₀ values are calculated which can be termed as the concentration of the antioxidant where it shows 50% of inhibition.

According to the results given in the table 1, cinnamon shows the highest inhibition with the requirement of 0.009 mg/ml to show a 50% inhibition. That totally agrees with the results obtained by ¹⁵ where he stated that cinnamon bark extracts show the highest radical scavenging activity in terms of the herbs and spices he evaluated.

Black cumin seeds cited the lowest IC₅₀ value with the requirement of 2.935 mg/ml to give 50% of inhibition. This result goes parallel with the total phenolic content as black cumin seeds showed the lowest TPC among the tested spices. But the result has been deviated from the results obtained by ¹⁷, where black cumin has shown 44% of inhibition at 0.5 mg/ml.

According to the results obtained, garlic needs 2.446 mg/ml in order to cause 50% inhibition of the DPPH radicals. These results are compatible with the results obtained by ¹⁶.

C. Determination of Total Antioxidant Capacity

Total antioxidant capacities (TAC) of garlic, cinnamon bark and black cumin seeds are given in the table 1 as Gallic acid equivalents, mg/g of the obtained extract.

Total antioxidant capacity/ Phosphomolybdenum assay is a spectrophotometric method in determination of total antioxidant activity. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte. The reduction yields a green phosphate/Mo(V) complex at acidic pH and that is detected spectrophotometrically at 695nm ¹⁴.

According to the results given in the table 1, cinnamon shows the highest antioxidant activity by yielding 149.15 mg GAE/mg of the extract which is significantly different from the other two. This is attributable with the other phytochemical results of cinnamon, which resulted highest phenolic content and highest radical scavenging ability.

In terms of black cumin seed, it has shown the lowest total antioxidant activity, moderate phenolic content and lowest free radical scavenging ability. Garlic yielded a lower total phenolic content but has shown a higher antioxidant capacity for phosphomolybdenum assay. Results obtained for TAC assay is agrees with the results obtained for the total phenolic content.

IV. CONCLUSION

Among cinnamon, garlic and black cumin seeds, cinnamon bark was cited with the highest TPC, radical scavenging activity and the total antioxidant capacity. Black cumin seeds

has shown the lowest occurrence except in the total phenolic content. Even though garlic contains comparatively lower phenolic content it has a higher radical scavenging activity and total antioxidant capacity than black cumin seeds.

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