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Turmeric and cinnamon dominate in antioxidant potential among four major spices

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Abstract

Antioxidant activity of sequential extracts of black pepper, ginger, turmeric and cinnamon was determined by DPPH assay, phosphomolybdate method and ferric reducing power method and compared with that of the synthetic antioxidant BHA. The results revealed that methanol extract of cinnamon has highest antioxidant potential followed by chloroform extract of turmeric. The antioxidant potential was also correlated with total phenol content.

Keywords: antioxidant activity, black pepper, cinnamon, ginger, total phenol content, turmeric

Abbreviations: 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), gallic acid equivalents (GAE), molar ascorbic acid equivalence (MAAE), phosphomolybdenum method (PM method).

Introduction

Reactive oxygen species (ROS), such as superoxide radicals, peroxyl radicals and hydroxyl radicals generated as a part of normal metabolic processes, causes oxidative damages of the biomolecules namely lipids, proteins and nucleic acids and triggers various chronic diseases such as coronary heart diseases, atherosclerosis, cancer and aging. Antioxidants by virtue of their capacity to inhibit oxidation processes help to combat these conditions. The commonly used antioxidants, namely, butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) and butylated hydroquinone (BHQ) are reported to have side effects such as liver damage and carcinogenesis (Sherwin *et al.* 1990). Due to consumer awareness and concerns about food safety, there is an increasing demand on natural antioxidants of plant origin. Plants contain various classes of phytochemicals with antioxidant properties *viz.*, phenolics, flavonoids, alkaloids, carotenoids, sterols and glucosinolates. Spices are excellent sources of phenolic compounds amd reported to show wide spectrum of pharmacological effects (Parthasarathy *et al.* 2008, Shan *et al.* 2005).

Major bioactive compound of black pepper (*Piper nigrum* L., Piperaceae), is an alkaloid, piperine, which shows its pharmacological impact on the nervous, neuromuscular and gastrointestinal systems (Katia *et al.* 2013). The principal active component of ginger (*Zingiber officinale* Rosc., Zingiberaceae), 6-gingerol, is a

phenolic compound. 6-Gingerol and its derivatives have wide range of medicinal properties like antimicrobial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer activities (Bartley *et al.* 2000; Dugasani *et al.* 2009; Grzanna *et al.* 2005; Shukla & Singh 2007). Turmeric (*Curcuma longa* L. Zingiberaceae), a widely studied Indian spice, is used in traditional medicines from ancient times. It has a broad spectrum of medicinal properties, which include anticarcinogenic, antioxidant, anti-inflammatory, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antimutagenic, antiulcer, hypotensive and hypocholesteremic activities (Warrier *et al.* 1995). The major constituent which contributes these properties of turmeric is the pigment, curcumin. Cinnamon (*Cinnamomum verum* Syn. *C. zeylanicum*, Lauraceae) with cinnamaldehyde as the chief constituent possesses significant antidiabetic, antiallergic, antipyretic, antiulcerogenic, anaesthetic, vasodilatory, antitumor, antifungal, cytotoxic and antimutagenic activities (Kurokawa *et al.* 1998; Shaughnessy *et al.* 2001). In the present study the antioxidant potential of sequential extracts of four Indian spices namely black pepper, ginger, turmeric and cinnamon was evaluated in relation to their phenol content.

Materials and methods

Plant sample collection

All the spice samples were collected from authentic sources focusing on a single variety. Berries of black pepper cv. Panniyur-5 were collected from Pepper Research Station, Panniyur, Kerala; ginger (*IISR Varada*) and turmeric (*IISR Prathibha*) rhizomes were collected from ICAR-Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi, Kozhikode and cinnamon bark from Cheemeni Estate, The Plantation Corporation of Kerala Ltd., Kannur District, Kerala.

Preparation of spice extracts

Black pepper berries, cinnamon bark, peeled ginger rhizomes and cured turmeric rhizomes were dried to a moisture level of 10% and powdered in a cyclotech mill. The powdered spice (500g each) was successively extracted with hexane, chloroform and methanol in a soxhlet apparatus for 30 h. The extracts were filtered and evaporated to dryness using rotary evaporator. The yield of solvent-free extracts was recorded and the extracts were stored at 4°C till further use.

Antioxidant potential

DPPH radical scavenging assay

Each extract (0.5 g) was dissolved in 100 mL methanol (5000 µg mL^{-1}) and used as the stock solution. Working standards of varying concentration were prepared and used for the assays. The hydrogen atom donating property of the spice extracts were determined by the decolourization of the methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Blois 1958). Working standards of various extracts was added to 1 mL of 0.004% DPPH (in methanol) the reaction mixture was made up to 5 mL with methanol and incubated in dark at room temperature for about 30 min. After incubation, the decrease in absorbance of the mixture was read at 517 nm and the percent inhibition was calculated. BHA was used as standard. The DPPH radical scavenging activity was expressed as IC_{50} value, which indicate the effective concentration of the extract for scavenging 50% of DPPH free radicals.

Phosphomolybdenum assay

Total antioxidant capacity of the extracts was estimated based on the reduction of molybdenum - VI to molybdenum - V by the sample and the subsequent formation of a green phosphate/molybdenum - V complex at acidic pH (Prieto *et al.* 1999). Varying concentration of the extracts was added to 1 mL reagent solution and incubated in a boiling water bath at 95°C for 90 min. Subsequently, the solution was made up to 4 mL using methanol and the absorbance was read at 695 nm. Ascorbic acid was used as the standard. The antioxidant potential was expressed in terms of MAAEg^{-1} of extract.

Ferric reducing power

The compounds with antioxidant properties tend to form a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride. The yellow color of the test solution changes to various shades of green and blue depending on the reducing power of the active compound. The ferric reducing potential is based on the principle of increase in the absorption of the reaction mixture which indicates increased antioxidant potential (Oyaises 1986).

Different concentrations of the aqueous solution of extracts (0.5 mL) were mixed with (2.5 mL) phosphate buffer (0.2 M, pH = 6.6) and (2.5 mL) potassium ferricyanide ($[K_3Fe(CN)_6]$, 1% w/v). The reaction mixtures were incubated at 50°C for 30 min. Trichloroacetic acid (10% w/v, 2.5 mL) was added to the reaction mixture. An aliquot of solution (2.5 mL) was mixed with 0.5 mL of $FeCl_3$ (0.1% w/v,) and 2.5 mL distilled water was added. The absorbance of the sample was measured at 700 nm. Ascorbic acid was used as the standard. The antioxidant potential of extracts was expressed as MAAE g^{-1} extract.

Total phenol content:

The total phenol content of each extract was assessed by Folin-Ciocalteu phenol reagent method (Singleton *et al.* 1999). Varying concentrations of the extracts were made up to 3 mL using reagent grade water to which the Folin-Ciocalteu phenol reagent (0.5 mL) was added and incubated at room temperature for 3 minutes. To the reaction mixture, 2 mL Na_2CO_3 solution (20%) was added and the mixture was kept in boiling water bath for about 1min, cooled and the absorbance of the solution was measured at 760 nm. Gallic acid was used as standard. The phenolic content was expressed as mg gallic acid equivalents per gram of the extract.

Statistical analysis

The data were analyzed using Excel (Microsoft Inc.) and SPSS version 5.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA) and Duncan's multiple-range test ($P<0.05$). Pearson's correlation was used to determine the correlation of antioxidant potential of chloroform and methanol extracts by DPPH free radical-scavenging activity, phosphomolybdenum method and ferric reducing power assay on total phenolic content. Data obtained were reported as mean ± standard deviation.

Results and discussion

The recovery of solvent extracts from the powdered black pepper berries, cinnamon bark and rhizomes of ginger and turmeric is given in Table 1. The antioxidant potential of the various spice extracts are indicated in Table 2. DPPH Free radical scavenging activity of spice extracts was expressed in terms of IC_{50} values, which varied between 11.9 µg mL^{-1} to 1507.3 µg mL^{-1} for different spice extracts. The highest activity was observed with methanol extract of cinnamon with IC_{50} value of 11.9 µg mL^{-1} which was followed by chloroform extract of turmeric (IC_{50} =18.2 µg mL^{-1}) whereas IC_{50} value of BHA was 5.4 µg mL^{-1}. Statistical analysis of the data indicated that the free radical scavenging activity of methanol extract of cinnamon and chloroform extract of turmeric were not significantly different and free radical scavenging activity of the sequential extracts was in the order, methanol extract of cinnamon, chloroform extract of turmeric > chloroform extract of ginger, hexane extract of turmeric, hexane extract of ginger > methanol extract of black pepper > methanol extract of ginger > chloroform extract of cinnamon, chloroform extract of black pepper > methanol extract of turmeric > hexane extract of cinnamon > hexane extract of black pepper. Studies by Oyas *et al.* (2013) using ethyl acetate extract of cinnamon, turmeric and ginger revealed that the cinnamon extract had

Table 1. Recovery (%) of the various spice extracts

Spice	Hexane	Chloroform	Methanol
Black pepper	10	6.0	4.0
Cinnamon	2.8	0.8	22.1
Ginger	3.0	1.2	4.2
Turmeric	9.0	7.6	9.2

maximum DPPH scavenging activity followed by turmeric and ginger extracts.

Antioxidant activity of the extracts by phosphomolybdenum assay varied from 0.27-2.99 MAAE g^{-1} of extract where as that of BHA was 3.68 MAAE g^{-1}. The chloroform extract of turmeric showed the highest antioxidant potential by this method (2.99 MAAE g^{-1} of extract) followed by the methanol extract of cinnamon (2.34 MAAE g^{-1} of extract). BHA showed 3.68 MAAE g^{-1} by phosphomolybdenum method. Statistical analysis revealed the following order of antioxidant by activity by this method: chloroform extract of turmeric > methanol extract of cinnamon > chloroform extract of cinnamon, hexane extract of turmeric, chloroform extract of ginger > methanol extract of turmeric > methanol extract of black pepper, chloroform extract of black pepper, hexane extract of cinnamon, hexane extract of ginger > methanol extract of ginger > hexane extract of black pepper. There was no significant difference among the antioxidant potential of chloroform extract of cinnamon, hexane extract of turmeric, and chloroform extract of ginger. Similarly, methanol extract of black pepper, chloroform extract of black pepper, hexane extract of cinnamon and hexane extract of ginger were on par with respect to their antioxidant activity.

The antioxidant potential of the spice extracts by FRP method varied between 0.20-1.56 MAAE g^{-1} of extract with 2.34 MAAE g^{-1} for BHA. Highest antioxidant activity was obtained with methanol extract of cinnamon which was followed by chloroform extract of turmeric (1.47 MAAE g^{-1} of extract); but statistical analysis of the data indicated that these two were not

Table 2. Antioxidant potential of spice extracts

Spice	Extract	DPPH Assay IC$_{50}$ (µg mL^{-1} of extract)	PM assay (MAAE g^{-1} of extract)	FRAP method (MAAE g^{-1} of extract)	TPC (mg GAE g^{-1} of extract)
Black pepper	Hexane	1507.3^{h}	0.27^{g}	0.28^{c}	16.03^{f}
	Chloroform	195.7^{e}	0.90^{e}	0.73^{b}	25.97^{f}
	Methanol	60.8^{c}	0.93^{e}	0.74^{b}	38.24^{e}
Cinnamon	Hexane	689.2^{g}	0.86^{e}	0.27^{c}	33.33^{f}
	Chloroform	166.3^{e}	1.31^{c}	0.37^{c}	35.00^{e,f}
	Methanol	11.9^{a}	2.34^{b}	1.56^{a}	244.67^{a}
Ginger	Hexane	36.2^{b}	0.82^{e}	0.34^{c}	103.67^{c}
	Chloroform	31.3^{b}	1.25^{c}	0.45^{c}	125.00^{c}
	Methanol	136.0^{d}	0.43^{f}	0.20^{c}	72.66^{d}
Turmeric	Hexane	35.7^{b}	1.28^{c}	0.38^{c}	120.00^{c}
	Chloroform	18.2^{a}	2.99^{a}	1.47^{a}	158.67^{b}
	Methanol	325.3^{f}	0.95^{d}	0.41^{c}	47.00^{e}
BHA		5.4	3.68	2.34	

Values with the same superscript within each column are not significantly different (P<0.05).

significantly different. By FRP method the antioxidant potential of sequential extracts was as follows: methanol extract of cinnamon, chloroform extract of turmeric > methanol extract of black pepper, chloroform extract of black pepper > chloroform extract of ginger, methanol extract of turmeric, hexane extract of turmeric, chloroform extract of cinnamon, hexane extract of ginger, hexane extract of black pepper, hexane extract of cinnamon, methanol extract of ginger. The data showed superior antioxidant potential of chloroform extract of turmeric and methanol extract of cinnamon among the 12 extracts and the least activity was observed with hexane extract of black pepper. However the antioxidant potential of all four spices tested are lower than that of the synthetic antioxidant BHA.

Total phenolic content of the spice extracts, varied between 16.03-244.67 mg GAE g^{-1} extract. The results showed that the methanol extract of cinnamon had the highest total phenol content (244.67 mg GAE g^{-1} extract) followed by chloroform extract of turmeric (158.67 mg GAE g^{-1} extract). The antioxidant potential of the spice extracts showed direct correlation with total phenolic content. Total phenol content of chloroform extracts showed significant correlation with DPPH assay(r = -0.98), phosphomolybdenum method (r=0.78) and FRP (r=0.61). Total phenol content of methanol extracts showed significant correlation with DPPH (r= -0.58), phosphomolybdenum method (r= 0.90) and FRAP (r= 0.87). Similar correlation between antioxidant activity and total phenol content was reported by Cai *et al.* (2004), Shan *et al.* (2005), Wu *et al.* (2006), and Wong *et al.* (2006) also. Spices like cinnamon and turmeric are very important not only for their culinary property but also for their antioxidant mediated activity. Further investigations are warranted to pinpoint the specific compounds responsible for the antioxidant activity of these extracts and their *in vivo* efficacies.

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