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Comparative evaluation of various total antioxidant capacity assays applied to phytochemical compounds of Indian culinary spices

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Abstract

Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicines and food preservatives. The purpose of this study was to evaluate the total content of polyphenols, flavonoid, tannins and their correlation to antioxidant activity of methanolic and aqueous extracts of spices. Cardamom, coriander seeds and dried bay leaves were used to prepare extracts and iron(III) reduction, 1,1-diphenyl-2-picrylhydrazyl radical-scavenging, hydrogen peroxide , superoxide and nitric oxide radical scavenging , reducing power were assayed as antioxidant capacity. Although bay leaves showed greater amount of phenols and high antioxidant activity, cardamom and coriander are also good sources of flavonoid and scavengers of free radicals. Both extracts of these spices are promising alternatives to synthetic substances as food ingredients with antioxidant activity.

Introduction

Oxidative stress has been widely implicated in biomedical sciences during the last 20 years. It significantly participates in the pathophysiology of highly prevalent diseases such as diabetes, hypertension, atherosclerosis, acute renal failure, Alzheimer and Parkinson diseases, among others. The metabolism of oxygen by cells generates potentially deleterious reactive oxygen species (ROS). Under normal conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination. However, an imbalance between pro-oxidants and antioxidants results in oxidative stress. Increased ROS levels in the cell have a substantial impact either leading to defective cellular function, aging or disease. Antioxidants have become rather popular lately (Ramon, 2009). Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicines and food preservatives (Milovanović *et al*., 2009; Šarić *et al*., 2009; Škrinjar *et al.*, 2009). Presently, there is an increasing interest both in the industry and in the scientific research of spices and aromatic herbs because of their strong antioxidant properties (Kabić *et al.*, 2008).

In this study, the potential beneficial role of antioxidants in Indian spices is discussed in the light of its phytochemical content and by using free radical scavenging assays like DPPH, FRAP, SO, NO and H_2O_2. Bay leaves (*Cinnamomum tamala*) refer to the aromatic leaf of the bay family, Lauraceae. Fresh or dried bay leaves are used in cooking for their distinctive flavor and fragrance. Bay leaf contains eugenol, which has anti-inflammatory, antifungal, antibacterial properties. It has also been used to treat rheumatism, amenorrhea, and colic (Masoumeh *et al.*, 2005).

Coriander (*Coriandrum sativum*) is an annual herb in the family Apiaceae. Coriander has been used as a folk medicine for the relief of anxiety and insomnia. Experiments in mice support its use as an anxiolytic (Singh *et al.*, 2007). Seeds are used as a drug for indigestion, against worms, rheumatism and pain in the joints. Recent studies have also demonstrated hypoglycaemic action and effects on carbohydrate metabolism (Chithra *et al*., 2000; Craig *et al*., 1999). Seeds of cardamom (Elettaria cardamomum) from family Zingiberaceae, are used as the spice ingredient in food and is very popular in Indian cuisine. Cardamom contains flavonoid like quercetin, kaempferol, luteolin and pelargonidin that are responsible for its antioxidant activity (Sultana *et al*., 2010). Cardamom efficiently reduces blood pressure, enhances fibrinolysis without significantly altering blood lipids or fibrinogen level in stage 1 hypertensive individuals. (Verma *et al*., 2009).

Although antioxidant activity has been demonstrated in spices, only a limited number of tests have been performed for its characterization. Information pertaining to phytochemicals in these spices is also scarce. Hence, the present research was designed to determine the phytochemical constituents and *in vitro* antioxidant activity of methanolic

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and aqueous extracts through a number of testing methods.

Materials and Methods

Plant material and Preparation of Extracts

Cardamom, coriander seeds and dried bay leaves were collected and authenticated. The dried samples were then ground to fine powder. 30 g of the dry powder was weighed and was used for extract preparation. Extracts for the plants were prepared using both methanol (methanolic extract) and distilled water (aqueous extract) as solvents. Using 150 ml of the respective solvent, 30 g of the dry powder was ground to a paste in mortar and pestle and was filtered twice through Whatman filter paper. The resulting filtrate was collected in a beaker and was subjected to evaporation in a rotary evaporator for 10 min at 100°C (for aqueous extraction) and 60°C (for methanolic extraction). The extracts were diluted appropriately before use.

Estimation of phytochemical constituents

Estimation of total phenol content (TPC)

The total phenol content was determined by Folin-Ciocalteu reagent method (McDonald *et al*., 2001) and expressed in terms of gallic acid equivalent (mg/g) (Chanda *et al.*, 2009).

Estimation of total flavonoids (TF)

The total flavonoid content was determined by aluminum chloride method (Chang *et al.*, 2002) and expressed in terms of quercetin equivalent (mg/g).

Estimation of sugars

Estimation of sugars in the extract was done by DNSA method (Mohun *et al.*, 1962). Maltose is a reducing sugar which will reduce 3,5 - dinitro salicylic acid (DNSA) to 3 - amino - 5 - nitrosalicylic acid in alkaline medium that is orange colored and absorbance was measured at 525 nm. Sugar content was expressed in terms of maltose equivalent (mg/g).

Estimation of tannins

The tannin content was determined by Folin-Ciocalteu reagent method (Chanda *et al*., 2009) and expressed in terms of tannic acid equivalent (mg/g).

Evaluation of antioxidant activity

α, α-diphenyl- β-picryl-hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1-diphenyl-2-picryl-hydrazyl by the method of McCune and Johns (McCune *et al*., 2002). This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the nonradical form DPPH-H. The absorbance was measured at 517nm (Chanda *et al*., 2009). DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g).

Nitric oxide (NO) radical scavenging assay

Nitric oxides generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reagent at 540 nm (Green *et al*., 1981; Chanda *et al.*, 2009). NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ). Decrease in the absorbance (593 nm) is proportional to the antioxidant content. (Chanda *et al.*, 2009). The antioxidant capacity was expressed in terms of ascorbic acid equivalent (mg/g).

Estimation of reducing power (RP)

The reducing power was determined by the method of Athukorala *et al.* (2006). Reducing power may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. Absorbance was measured at 700 nm (Chanda *et al*., 2009). RP was expressed in terms of ascorbic acid equivalent (mg/g).

Superoxide anion (SO) radical scavenging assay

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski (1998). In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture (Chanda *et al.*, 2009). SO anion scavenging activity was expressed in terms of Gallic acid equivalent (mg/g).

Hydrogen peroxide (H_2O_2) radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of

Ruch *et al*. (1989). H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH^-). Decrease in absorbance at 230 nm is determined. (Chanda *et al*., 2009). H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

Statistical analysis

The results are expressed as mean ± SD. All measurements were replicated three times.

Results and Discussion

Total phenolics, flavonoids, sugar and tannin content of coriander seeds, cardamom seeds and bay leaf extracts are presented in Table 1. Results showed that extracts contained variable amounts of these compounds. The amount of total phenolics was highest in methanolic extract of bay leaves. Bay leaves are rich in phytochemicals like tannins, phenols, parthenolides, eugenol, linalool, monoterpenoides, flavonoid which contribute to its antioxidant potential (Shan *et al*., 2005).

Coriander seeds also showed a good content of phenolics. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Yanli *et al.*, 2007).

Reducing sugar content was highest in coriander (methanolic) extract, followed by cardamom (aqueous) extract. Like phenols, polysaccharides also exhibit varied bio-activities such as antitumor, anticancer, antiviral, antibacterial, antifungal, anticoagulant and immunological activity as reported by (Xu *et al.*, 2009).

Tannin is actually an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. This tannin-protein complex can provide persistent antioxidant activity. The tannin content was highest in bay leaves (Ypuwei *et al*., 2008). Tannins act as antioxidants action by scavenging free radicals, chelating transition metals, inhibiting pro-oxidative enzymes and by inhibiting lipid peroxidation (Koleckar *et al.*, 2008).

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. Data of comparative analysis of antioxidant activities of samples are presented in Table 2. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is widely used to assess the radical scavenging activity of antioxidant compounds (Thaipong *et al.*, 2006; Khalaf *et al*., 2008). Both cardamom and bay leaf extracts displayed striking DPPH radical scavenging activities that might be attributed to their hydrogen donating ability (Zhao *et al*., 2006).

NO is a potent diffusible free radical generated by the endothelial cells and macrophages, which is a mediator of various physiological processes. The reduction of NO radical by cardamom seeds and bay leaves was found to be higher in aqueous extract than in methanolic extract (Table 2) as also seen in the results of Seori Jin *et al.* (2011). Coriander showed less NO scavenging activity then cardamom but higher than cinnamom methanolic extract. Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds (Sonawane *et al.*, 2010).

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ (Benzie *et al.*, 1996). The FRAP in bay leaves (methanolic) was found to be highest among all extracts. There is a significant difference between FRAP in both extracts of bay leaves (Table 2). The FRAP activity is correlated to high phenolic and flavonoid compounds namely quercetin, kaempferol and quercetrin in bay leaves. Our results are in accordance with that of Duan *et al.* (2007) who have proposed cinnamom leaves as a natural antioxidant source and alternative to synthetic antioxidants. Coriander and cardamom also show significant FRAP activity.

Reducing power is generally associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain through donating a hydrogen atom (Duan *et al*., 2007). Cardamom showed maximum reducing power and good correlation exists between reducing power, DPPH radical scavenging activity and total phenol content (Padmakumari *et al*., 2010). Superoxide anion is a weak oxidant; still it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Chanda *et al*., 2009).

Cinnamom (methanolic) extract exhibited excellent superoxide anion scavenging activity which is comparable to cardamom. SO scavenging activity is associated to total flavonoids content (Chen *et al.*, 2006; Chanda *et al*., 2009). Flavonoid molecule with polyhydroxylated substitution on ring A or B

Table 1. Phytochemical constituents in *Coriandrum sativum*, *Ellettaria cardamomum* and *Cinnamom tamala* extracts

Tests	Standard equivalent in methanolic extract (mg/g)			Standard equivalent in aqueous extract (mg/g)		
	Coriandrum sativum	*Ellettaria cardamomum*	*Einnamomum tamala*	*Coriandrum sativum*	*Ellettaria cardamomum*	*Cinnamomum tamala*
Total phenol content	1.36±0.15	1.25±0.17	7.24±1.09	3.14±0.12	0.55±0.08	0.16±0.05
Total flavonoids	0.34±0.07	2.85±0.11	0.5±0.07	0.78±0.01	1.8±0.11	0.54±0.07
Sugar content	10.6±0.41	5.5±0.43	1.38±0.58	15.16±0.76	3.15±0.45	0.72±0.07
Tannin content	0.6±0.05	0.55±0.08	4.53±0.83	1.4±0.03	0.22±0.07	1.4±0.07

(The results obtained were expressed as Mean ± S.D. of triplicates).
TPC expressed as mg of gallic acid equivalent /g of sample
TF expressed as mg of quercetin equivalent /g of sample
Sugar content expressed as mg of maltose equivalent /g of sample
Tannin content expressed as mg of tannic acid equivalent /g of sample

Table 2. Antioxidant activity of *Coriandrum sativum, Ellettaria cardamomum* and *Cinnamom tamala* extracts

TESTS	Standard equivalent in methanolic extract (mg/g)			Standard equivalent in aqueous extract (mg/g)		
	Coriandrum sativum	*Ellettaria cardamomum*	*Cinnamomum tamala*	*Coriandrum sativum*	*Ellettaria cardamomum*	*Cinnamomum tamala*
DPPH scavenging assay	6.9 ± 0.01	63.0 ± 1.5	8.23 ± 0.05	2.86 ± 0.07	61.75 ± 3.03	6.9 ± 0.05
NO radical scavenging assay	5.66 ± 0.17	9.35 ± 0.31	2.46 ± 0.05	4.7 ± 0.07	12.05 ± 0.75	5.96 ± 0.05
FRAP assay	7.53 ± 0.14	4.6 ± 0.08	30.34 ± 1.68	6.9 ± 0.05	4.55 ± 0.99	11.12 ± 0.14
Reducing power assay	1.2 ± 0.1	6.0 ± 0.70	0.57 ± 0.21	0.23 ± 0.03	6.75 ± 0.46	1.2 ± 0.17
SO radical scavenging assay	42.6 ± 0.28	66.25 ± 0.32	68.3 ± 1.52	32.6 ± 0.26	64.95 ± 0.07	41.6 ± 0.26
H_2O_2 radical scavenging assay	1.86 ± 0.17	27.95 ± 3.06	8.36 ± 0.64	1.73 ± 0.24	35.5 ± 0.25	6.26 ± 0.67

(The results obtained were expressed as Mean ± S.D. of triplicates)
DPPH, NO, FRAP, RP, H2O2 expressed as mg of ascorbic acid equivalent /g of sample
SO expressed as mg of gallic acid equivalent /g of sample

and a free 3-hydroxyl substitution could present the superoxide scavenging activity (Siddhuraju *et al*., 2002; Bravo *et al*., 1998). Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water (Nabavi *et al*., 2008; Ebrahimzadeh *et al*., 2009). The H_2O_2 scavenging activity was estimated to be higher in the aqueous cardamom extract then in bay leaves which is correlated to phenol content of these extracts (Duan *et al*., 2007).

Conclusion

There are many reports of herb and spice extracts being used in ayurvedic literature which are directly or indirectly used for the preparation of many modern drugs. There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently, used natural and synthetic antioxidants. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. and render spices and some herbs or their antioxidant components as preservative agents in food.This study provided evidence on the potential health benefits of spices of Indian cuisine.

In our study, a significant linear correlation was found between the concentration of phenolic compounds and the antioxidant activity of extracts from different spices. Among the three spice investigated we found that cardamom exhibited maximum antioxidant activity in all *in vitro* models. Bay leaf and coriander were also rich in antioxidants. In the light of these findings it can be stated that use of natural antioxidants occurring spices in the Indian diet, or their extracts, is a viable alternative to synthetic antioxidants without any concern. Further investigation of individual compounds, there *in vivo* antioxidant activities and in different antioxidant mechanisms is warranted.

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