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

Deepa, G., Ayesha, S., Nishtha, K. and Thankamani, M.

Department of Biotechnology and Bioinformatics, Padmashree Dr. D. Y. Patil University, Sector 15, CBD Belapur, Navi Mumbai, Maharashtra, India

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 H₂O₂. 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-pircryl-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 Fe³⁺ to Fe²⁺ 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 (H₂O₂) radical scavenging assay**

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of
Ruch et al. (1989). H$_2$O$_2$ is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH$^\cdot$). Decrease in absorbance at 230 nm is determined. (Chanda et al., 2009). H$_2$O$_2$ 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, monoterpeneoildes, 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 cinnamon 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 cinnamon 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.
and a free 3-hydroxyl substitution could present the superoxide scavenging activity (Siddhuraju et al., 2008; Ebrahimzadeh et al., 2007). Scavenging of \( \text{H}_2\text{O}_2 \) by extracts may be attributed to their phenolics, which can donate electrons to \( \text{H}_2\text{O}_2 \) thus neutralizing it to water (Nabavi et al., 2008; Ebrahimzadeh et al., 2009). The \( \text{H}_2\text{O}_2 \) 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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Table 1. Phytochemical constituents in *Coriandrum sativum*, *Ellettaria cardamomum* and *Cinnamomum tamala* extracts

<table>
<thead>
<tr>
<th>Tests</th>
<th>Standard equivalent in methanolic extract (mg/g)</th>
<th>Standard equivalent in aqueous extract (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Coriandrum sativum</em></td>
<td><em>Ellettaria cardamomum</em></td>
</tr>
<tr>
<td>Total phenol content</td>
<td>1.36±0.15</td>
<td>1.25±0.17</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>0.34±0.07</td>
<td>2.85±0.11</td>
</tr>
<tr>
<td>Sugar content</td>
<td>10.6±0.41</td>
<td>5.5±0.43</td>
</tr>
<tr>
<td>Tannin content</td>
<td>0.64±0.05</td>
<td>0.55±0.08</td>
</tr>
</tbody>
</table>

(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 *Cinnamomum tamala* extracts

<table>
<thead>
<tr>
<th>TESTS</th>
<th>Standard equivalent in methanolic extract (mg/g)</th>
<th>Standard equivalent in aqueous extract (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Coriandrum sativum</em></td>
<td><em>Ellettaria cardamomum</em></td>
</tr>
<tr>
<td>DPH scavenging assay</td>
<td>6.9±0.01</td>
<td>63.1±1.5</td>
</tr>
<tr>
<td>NO radical scavenging</td>
<td>5.66±0.17</td>
<td>9.35±0.31</td>
</tr>
<tr>
<td>FRAP assay</td>
<td>7.53±0.14</td>
<td>4.6±0.08</td>
</tr>
<tr>
<td>Reducing power assay</td>
<td>1.2±0.1</td>
<td>6.0±0.70</td>
</tr>
<tr>
<td>SO radical scavenging</td>
<td>42.6±0.28</td>
<td>66.25±0.32</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 ) radical scavenging</td>
<td>1.8±0.17</td>
<td>27.95±3.06</td>
</tr>
</tbody>
</table>

(The results obtained were expressed as Mean ± S.D. of triplicates)

DPPH, NO, FRAP, RP, \( \text{H}_2\text{O}_2 \) expressed as mg of ascorbic acid equivalent /g of sample

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use of laboratory facilities and technical assistance.

References


Singh, G., Maurya, S., Lampasona, M. P. and Catalan, C.


