Comparative analysis of spices for their phenolic content, flavonoid content and antioxidant capacity

Dr. Deepshikha Gupta
Amity Institute of Applied Sciences
Amity University Uttar Pradesh
Sector 125, Noida-201301, India

Abstract: Nine different spices used in Indian kitchen were analysed for their total phenolic contents (TPC), total flavonoid content (TFC) and antioxidant activity AOA (using DPPH assay and FRAP assay). The spices used were cinnamon (Cinnamomum verum), black pepper (Piper nigrum), ajwain (Trachyspermum ammi), cumin (Cuminum cyminum), black cumin (Nigella sativa), coriander (Coriander sativum), fennel (Foeniculum vulgare), star anise (Illicium verum) and clove (Syzygium aromaticum). The methanolic extracts of the above spices were used for all the analysis. The TPC showed the following order: maximum for cloves>cinnamon>ajwain>cumin>black pepper>fennel>coriander>black cumin. The highest TFC obtained was in clove>cinnamon>ajwain>black cumin>star anise>fennel>coriander>black pepper. The spice showing maximum AOA was again clove followed by cinnamon. The results showed that cloves and cinnamon have excellent AO potential and further studies can be carried out to utilize their potential.

Keywords: Spices, antioxidant activity, total phenolic content, total flavonoid content, DPPH assay and FRAP assay.

I. Introduction

Spices are very integral part of both vegetarian and non-vegetarian Indian cooking. They are common food additives that impart flavour and aroma. A common Indian kitchen with onion, garlic, ginger, turmeric, tejpat, coriander, pepper, Ajwain, Jeera, tea, tulsi and neem leaves etc is actually a small herbal medical store. Spices can be the buds (cloves), bark (cinnamon), roots (ginger), berries (peppercorns), aromatic seeds (cumin) and even the stigma of a flower (saffron) [1]. The use of synthetic food antioxidant additives such as butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), and propyl gallate is under increasing regulatory scrutiny, and so attention is turning to the possibility that “natural” antioxidants may replace them for at least some food application [2]. Spices rich in antioxidants have been used for centuries to delay oxidative deterioration of foods during storage or cooking. They are a potential source of natural antioxidants that can be used for food preservation; as well they add flavour and aroma to food. Some of the active antioxidant components in spices include carnosic acid, carnosol, rosmarinic acid, thymol, carvacrol, 6-gingerol, 6-shogaol, zingerone, curcumin, capsaicin, vanillin, eugenol, caffeic acid, and ferulic acid [3].

We have chosen nine commonly used spices available commercially. Most of the biological activities shown by plant products are due to the presence of secondary plant metabolites like phenolics and flavonoids. Methanolic extract of powdered spice samples were used for analysis as methanol is the best solvent for phenolics.

The active constituents like phenolics, flavonoids, terpenes, tannins present in the spices selected are mainly responible for their activities. Star anise contains anethole, an ingredient responsible for its characteristic flavour, is the primary precursor for anti influenza drug Tamiflu [4]. The flavour of cinnamon is due to its aromatic oil containing 90% cinnamaldehyde, other compounds being ethyl cinnamate, eugenol, linalool, methyl chavicol and β-caryophyllene mainly contributes to its activity. Cinnamon powder is used as traditional medicine against herpes virus had an effect on HIV-1 and a significant effect on controlling type-2 diabetes by controlling the blood glucose levels [5]. Black pepper contains piperine and other components which imparts it spiciness and is believed to cure illness such as constipation, diarrhoea, gangrene, indigestion, liver and lung problems, joint pain, tooth problems etc.[6]. Ajwain is used as home remedy for stomach ache and contains thymol as the active ingredient. The distinctive flavour and aroma of Cumin seed is due to the presence of
cuminaldehyde, cuminic alcohol, substituted pyrazines and terpenes [7]. Nigella contains thymoquinone, an antineoplastic effect on cultured human cell lines. It also has protective antioxidant and antiinflammatory effects and promotes apoptosis of cancer cells. Other constituents of nigella oil are nigellone, melanthin, nigilin, tannins and damascanne [8]. Fresh coriander leaves contains antioxidants that can delay spoilage of food seasoned with spice. Coriander oil contains 60-65% coriandroil, pinene, geranial and traces of phellandrene, dipentene, terpinene, cymene and borneol. This has antibacterial property against Salmonella choleraesuis. It has diuretic and carminative properties. It is also used for treatment of type 2-diabetes and has significant hypolipidaemic effect in lowering of cholesterol and triglycerides [9]. Cloves contain 70% eugenol and other major constituents being β-caryophyllene, vanillin, crategolic acid, tannins and flavonoids. It is used in dentistry as anodyne, as carminative and anthelmintic [10]. Fennel seeds are also widely used for culinary purpose. Its active ingredient is anethole which is used for its carminative action [11].

II. Materials and methods

2.1 Materials: All solvents used were of HPLC grade. DPPH, trolox, gallic acid, quercetin, TPTZ (2,4,6-tripyridyl-s-triazine) and ferrous sulphate were procured from Sigma Aldrich. Folin and ciocalteu’s reagent, sodium acetate, sodium hydroxide, glacial acetic acid, HCl, sodium nitrite and aluminium chloride used were of analytical grades. Distilled water used was double distilled deionised water. The centrifuge machine used was of REMI (20000 rpm). The UV-Visible spectrophotometer used was Shimadzu UV-1800 model. The vacuum evaporator used was made Khera Co. make.

The dried whole intact spices were procured from local market. They were finally powder by high speed grinder. 50 mg of each of the powdered spice was extracted with 10 ml of methanol and centrifuged at 6000 rpm for 10 minutes for three times. The supernatant extract were mixed and concentrated under reduced pressure to 10 ml. The extracts were not dried completely as overheating may lead to disintegration of many important metabolites.

2.2 Total phenolic content (TPC) Folin-Ciocalteu method was used to determine total phenolic content [12]. 1mM stock of gallic acid was prepared for the standard curve. 100 µL of methanolic extract was mixed with Folin reagent (5ml, 1:10 dilution with distilled water) and aqueous sodium carbonate (4 ml, 1M). The mixture was vortexed and allowed to stand for 15 minutes after which the blue colour of reduced forms of phosphomolybdic and phosphotungstic acids. The total phenolic content was determined spectrophotometrically at 765nm against reagent blank as reference. The standard curve was prepared by plotting the absorbance against concentration (50, 100, 150, 200, 250, 300 and 350 µL dilutions of gallic acid stock solution in (50% aq methanol) were prepared.

2.3 Total flavonoid content (TFC): In the determination of total flavonoids content, quercetin (50, 100, 150, 200, 250, 300 µL of 1mM stock solution in distilled water) was used for the standard curve. 100 µL of extract was taken in a 10 ml test tube containing 4 ml distilled deionized water and 0.3 ml 5% sodium nitrite. After 5 min 0.3 ml of 10% aluminium chloride was added. At 6th minute 2ml of 1M NaOH was added and total volume was made up to 10 ml with distilled water. The solution was mixed well and absorbance was measured against a prepared reagent blank at 415 nm. It was observed that the yellow colour and promotes apoptosis of cancer cells.

2.4 DPPH assay (2,2-diphenyl-1-picrylhydrazyl radical) DPPH−, is a stable free radical which decolourises in the presence of antioxidants. It is a stable nitrogen centered free radical, and its colour changes from violet to yellow when it is reduced by either the process of hydrogen or electron donation. The method used was suggested by Brand-Williams 1995 [13]. A stock solution of 300µM concentration of DPPH− radical was prepared in methanol. 5 µL of spice extract was treated with 100 µL of DPPH· solution and 200 µL of methanol. The dark purple colour of DPPH− was discharged and a yellowish solution was obtained upon acceptance of electron from antioxidant compound. The DPPH· absorbs at 515nm. Antioxidant capacity was evaluated in terms of trolox equivalent (400mM stock solution was prepared in methanol) Trolox showed linearity range from 20 µL-320 µL. Absorbance was checked immediately after 5 min., 1 hr and after 18 hours for the spice extract as the rate of reaction was slow for some samples. The percentage inhibition values for DPPH radical activity were calculated from the absorbance of the control (A0) and the sample (As) using eq. (1). Gallic acid was used as positive control. The analysis was done in triplicates.

% Inhibition = (A0 − As)/A0 x 100 (1)
2.5 FRAP assay The method used was suggested by Benzie and Strain, 1999 [14]. It utilises Fe(III)(TPTZ)₂Cl₂ or ferric 2,4,6-tripyridyl-s-triazine as oxidant which has a redox potential of 0.77 V. At low pH reduction of Fe(III) TPTZ complex to ferrous form (intense blue colour) can be monitored by measuring the change in absorption at 596 nm. The change in absorbance is directly related to the combined or total reducing power of the electron donating antioxidants present in reaction mixture. The method was modified and the reaction was evaluated at 0 min, 4 min and 10 min. FRAP reagent was freshly prepared by mixing 200 ml of acetate buffer (300mM, pH 3.6), 20 ml of TPTZ solution (10mM), 20 ml of FeCl₃ (20 mM) and 24 ml distilled water. The straw coloured solution so obtained was kept at 37°C until used. 2ml of the freshly prepared FRAP reagent was taken in a spectrophotometer cuvette, to it 30 μL of spice extract and 70 μL of methanol was added. Absorbance was observed at 596 nm immediately, after 4 min and after 10 min intervals against reagent blank. For the standard curve FeSO₄ solution dilution from 0.2mM to 1.6 mM concentration were prepared from the stock and processed in the similar way.

III. Results and Discussion

The total phenolic content is used to analyse tannins, anthocyanins, monomeric phenolic compounds and polymeric pigments present in the sample. Total phenols in the range of 20-400 μg/L was analysed using this protocol. Turbid samples were centrifuged or vortexed with 1-2 min standing time before analysis. A correlation between the phenolic content and antioxidant activity has been shown earlier by number of workers [15]. The amount of total phenolics was investigated by Folin-Ciocalteu method [12]. The total phenolic content was expressed as gallic acid equivalents (mg gallic acid/ml of extract). The order of performance for TPC observed was highest in cloves>cinnamon>ajwain>Star anise>cumin>black pepper>fennel>coriander>black cumin. Rice-Evans et al [15] and Brand-Williams et al [13] demonstrated that the antioxidant activities of polyphenolic compounds are largely dictated by their molecular structure. The evaluation of the antioxidant activity of plant phenolic extracts is limited to an estimation of the total antioxidant activity of the system. The total flavonoid content in spice extracts was also analysed using Aluminium chloride method and Quercetin as standard. The highest Total flavonoid content (TFC) obtained was in clove>cinnamon> ajwain>black cumin>star anise> fennel>coriander>black pepper. Spices Cinnamon, Cove, black pepper and ajwain showed significant DPPH radical scavenging activity while FRAP assay showed highest activity for cinnamon followed by cloves, Cumin and star anise. The spices high in phenolic content also showed significant antioxidant activity using DPPH and FRAP assays. The results obtained verified the correlation between phenolic content and antioxidant activity i.e., samples high in phenolic content also showed high assay values for DPPH and FRAP assays.

The results tabulated in table 1 and represented in figure 1-5 are mean values of triplicate experiments. Statistical analysis to check linearity was carried out in Origin 6.0. Table 1 shows the assay results obtained for the various spices. Figure 1 is a calibration plot for TPC using gallic acid as reference standard. Figure 2 is a calibration plot for TFC using quercetin as standard. Figure 3 indicates the ferrous sulphate standard curve for analysis of FRAP of spice extracts. The reaction time for various spices showed difference hence time dependence for this assays for checked at three time intervals (at 0 min, 4 min and 10 min). The curves in figure 4 showed that cinnamon and cloves has highest reactivity and does not show much change with elapse of time (3%). Other spices reacted slowly and showed significant change in activity (as great as 100 % in cumin). The results tabulated in table 1 and represented in figure 1-5 are mean values of triplicate experiments.

This research paper draws a conclusion that the methanolic extracts of Cinnamon and Cloves exhibited strong antioxidant properties owing to the presence of high phenolic and flavonoid contents in them. The high activity may be attributed to the presence of eugenol, linalool, methyl chavicol and β-caryophyllene in cinnamon and eugenol, β-caryophyllene, vanillin, crategolic acid, tannins and flavonoids in cloves. Further research is needed to evaluate the antioxidant activity of isolated components with maximum activity. Also work can be carried out to establish that the total antioxidant activity is a measure of all the constituents present together and acting together or their individual performances.

Table 1: The results obtained for TPC, TFC, AOA (DPPH and FRAP assays)

<table>
<thead>
<tr>
<th>S/ No</th>
<th>Spice</th>
<th>Common Names</th>
<th>Family</th>
<th>Total Phenolic content (equivalent to mM Gallic acid)</th>
<th>Total Flavonoid content (equivalent to mM Quercetin)</th>
<th>DPPH radical activity reduction (%)</th>
<th>FRAP assay (equivalent to mM of FeSO₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cinnamamum verum</td>
<td>Dalchini or</td>
<td>Lauraceae</td>
<td>279.64</td>
<td>357.56</td>
<td>67.67</td>
<td>5.859</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th></th>
<th>Plant Name</th>
<th>Common Name</th>
<th>Family</th>
<th>Total Phenolic Content</th>
<th>Total Flavonoid Content</th>
<th>Total Antioxidant Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Piper nigrum</td>
<td>Black pepper</td>
<td>Piperaceae</td>
<td>6.54</td>
<td>21.49</td>
<td>46.6</td>
</tr>
<tr>
<td>3</td>
<td>Trachyspermum ammi</td>
<td>Ajwain</td>
<td>Apiceae</td>
<td>169.56</td>
<td>278.53</td>
<td>33.97</td>
</tr>
<tr>
<td>4</td>
<td>Cuminum cyminium</td>
<td>Cumin</td>
<td>Apiceae</td>
<td>12.15</td>
<td>46.6</td>
<td>9.61</td>
</tr>
<tr>
<td>5</td>
<td>Nigella sativa</td>
<td>Black cumin</td>
<td>Ranunculaceae</td>
<td>1.08</td>
<td>60.63</td>
<td>0.27</td>
</tr>
<tr>
<td>6</td>
<td>Coriander sativum</td>
<td>Coriander</td>
<td>Apiceae</td>
<td>2.62</td>
<td>38.41</td>
<td>6.98</td>
</tr>
<tr>
<td>7</td>
<td>Foeniculum vulgare</td>
<td>Fennel</td>
<td>Apiceae</td>
<td>6.37</td>
<td>38.98</td>
<td>6.13</td>
</tr>
<tr>
<td>8</td>
<td>Illicium verum</td>
<td>Star Anise</td>
<td>Schisandraceae</td>
<td>28.75</td>
<td>41.54</td>
<td>10.62</td>
</tr>
<tr>
<td>9</td>
<td>Syzygium aromaticum</td>
<td>Clove</td>
<td>Myrtaceae</td>
<td>425.04</td>
<td>462.19</td>
<td>63.46</td>
</tr>
</tbody>
</table>

Figure 1: Standard curve for total phenolic content

Figure 2: Standard curve for total flavonoid content

Figure 3: FeSO\(_4\) standard curve for FRAP assay

Figure 4: Time dependence curve for FRAP assay

Figure 5: Standard curve for DPPH assay
IV. References


V. Acknowledgement

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