Determination of Total Phenolics, Antioxidant Capacity and Antimicrobial Activity of Selected Aromatic Spices

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ABSTRACT

In recent years, it has been acknowledged that many spices not only have properties that make food more pleasant and tastier but they also have important preservative and antioxidant properties. The antioxidant properties of many spices are well known, while their prooxidant properties less so.

The aim of this study was to perform a comparative evaluation of three selected spice (garlic, black pepper and red pepper), commercially available in Cluj-Napoca’s market, regarding their content in total phenolic compounds, antioxidant capacity and antimicrobial activity. The antioxidant aspects were evaluated by determination of the total phenolic content by the Folin-Ciocalteau method and by the antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl reagent (DPPH). The total phenolic content was between 3.38 and 0.925 mg GAE/1g, while the antioxidant capacity ranged between 18.41% and 10.9% from dry spice. Results showed that garlic extract has the most effective antioxidant capacity in scavenging DPPH radicals, while black pepper and red pepper were less active.

In the present study piperine was evaluated for its antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhumurium. Spices showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-15.5 mm.

Keywords: antibacterial, antioxidant activity, black Pepper (Piper nigrum L), phenolic compounds, garlic (Allium sativum), red Pepper (Capsicum Annum L).

INTRODUCTION

Herbs and spices were considered an important part of human diet and have been used for thousands of years in traditional medicine and also to enhance color, flavor and aroma of the food (Andradea et al., 2013). An important field of research today is the control of ‘redox’ status by consuming foods with high antioxidant properties. Natural antioxidants present in the diet increase the resistance to oxidative stress and they may have a substantial impact on human health. Phenolic compounds, especially phenolic acids, are present in considerable amounts in vegetables; thus they are an integral part of the human diet. Recently, they have received much attention since many epidemiological studies suggested that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovas-cular diseases, stroke and certain forms of cancer (Ghasemnezhad et al., 2011). Antimicrobial and antioxidant properties of
spices render them useful as preservative agents (Chatterjee et al., 2007).

Red pepper (*Capsicum annuum* L.) is an important agricultural crop, not only because of its economic importance, but also for its rich antioxidant content, mainly due to the fact that it is an excellent source of ascorbic acid, natural colours and other antioxidant compounds (Ghasemnezhad et al., 2011). These compounds show potential action against certain cancers, stimulate the immune system, prevent cardiovascular diseases and protect against age-related macular degeneration (Salim et al., 2012).

Black pepper is the most widely used spice and occupies a proud place in the cuisines of both West and East, in both vegetarian and non-vegetarian cooking. Black pepper contributes towards flavor, taste, antifungal, antibacterial and antioxidant properties, the predominating ones being taste and flavor, and hence pepper is a multifunctional spice (Ravindran et al., 2012). Black pepper is also used in skin care, muscle and joint pains, and in improving blood circulation and respiratory systems. The bioactive molecule, piperine, present in pepper has major pharmacological impacts on the nervous and neuromuscular systems, exercises sedative effect and helps in digestion (Andradea et al., 2013). Piperine is the major chemical constituent responsible for the bitter taste of the black pepper (Silva et al., 2013).

Garlic (*Allium sativum* L.) belongs to the vegetables of the *Allium genus*, it has a long history as being a food having a unique taste and odor. Nowadays, garlic has been not only widely used as antibacterial, antiviral, antifungal and antiprotozoal, but it also has beneficial effects on the cardiovascular and immune systems. Researchers showed that there are more than 200 components identified from garlic, such as vitamins, proteins, lipids, trace elements Se, flavonoids and at least 33 different organosulfur compounds. Modern scientific research has revealed that the wide variety of dietary and medicinal functions of garlic can be attributed to the sulfur compounds from garlic (Fei et al., 2015). Garlic extracts have been used to treat infections for thousands of years. Its typical pungent odor and antibacterial activity depends on allicin, which is produced by enzymatic hydrolysis of allin after cutting and crushing of the cloves (Palaksha et al., 2010). Studies suggested that diallyl trisulfide (DATS) was responsible for the anticancer effect of garlic. Moreover, garlic contains stable organosulfur compounds, flavonoids and polyphenols, which had potent antioxidant properties (Fei et al., 2015).

The aim of the present study was to characterize three selected spice (garlic, black pepper and red pepper), commercially available in Cluj-Napoca’s market, regarding their content in total phenolic compounds, antioxidant capacity and antibacterial activity.

**MATERIALS AND METHODS**

The analysed samples were purchased from local supermarkets (Cluj-Napoca, Romania). Red pepper and Black pepper samples were available in the dry form and garlic was used fresh.

**Plant extracts**

The methanolic plant extracts were obtained according to the method described by Muresan et al., 2012. For sample extraction, 1g of powdered material was extracted with 10 ml of methanol. The extract was separated and the residual tissue was re-extracted until the extraction solvents became colorless. The filtrates were combined in a total extract, which was dried by vacuum rotary evaporator at 40°C. The dry residues were redisolved in methanol and stored in a freezer at -20°C until analyzed.

**Total phenolic content**

The determination of the total phenolics content was performed using the Folin-Ciocalteu reagent, according to Muresan et al. (2012) and Kodama et al. (2010) with some modifications. A 0.25 mL of the methanolic extract obtained above were mixed with 0.12 mL of the Folin-Ciocalteu reagent and 1.8 mL of distilled water. After 5 minutes at room temperature, 0.34 mL of a sodium carbonate (Na₂CO₃) solution 7.5% was added and the mixture placed at room temperature for 2 hours. The absorbance were measured at 750 nm on a Shimadzu UV-1700 PharmaSpec spectrophotometer. A calibration curve was performed using different concentrations of standard gallic acid solutions (r² = 0.9997) and the concentration of TPC was expressed as mg GAE/g dried material.

**Determination of 2,2-diphenylypicrylhydrazil radical scavenging capacity (DPPH)**

Scavenging activities of the extracts on the stable free radical DPPH were assayed using the method adapted after Anesini et al. 2008.
A volume of 0.1 mL of an methanolic extracts were mixed with 0.9 mL distilled water and 3.9 mL methanolic DPPH solution. After 30 minutes incubation in darkness, the absorbance of each sample was measured at 515nm against a blank of methanol.

The percentage of DPPH was calculated by measuring the absorbance of the sample and applying the following equation: % of inhibition = \[1 - (A_s/A_0)] \times 100,\] where \(A_s\) is the absorbance of sample, and \(A_0\) is the absorbance of the DPPH solution.

**Evaluation of antimicrobial activity**

Antibacterial activity was determined using the disk diffusion test method according to the Clinical and Laboratory Standards Institute guidelines. The bacteria used in this study were gram-positive *S. aureus* ATCC 49444, grame-negative *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028. The inoculum was prepared to contain \(10^8\) CFU/mL by adjusting the suspension to match the McFarland No-0.5 turbidity standard according to National Committee for Clinical Laboratory Standards and dilutions were made that corresponding to a population of \(1-5 \times 10^5\) CFU/mL. For antibacterial testing, selective medium agar agar was used, thus: Baird Parker Agar (Oxoid); Pseudomonas Agar P (base) (Merck); Tryptone Bile X-glucuronide Agar (Oxoid) And Xylose Lysine Deoxycholate Agar (Oxoid). Then, 100 μL of each suspension bacteria were inoculated on the agar plate’s entire surface. The dried plant extracts were dissolved methanol to a final concentration of 50 mg/mL. Extracts were pipetted (45 μL) onto sterile paper discs (6 mm diameter) and placed onto the surface of inoculated agar plates. All the plates were incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the zone of inhibition in millimetres. All experiments were done in duplicates.

**RESULTS AND DISCUSSION**

Results of the colorimetric analysis of total phenolics are given in Table 1 and the free radical scavenging activities of the obtained extracts from garlic, black pepper and red pepper are given also in Table 1.

The highest concentration in phenolic compounds was determined for *Piper nigrum* L (338 mg GAE/100g), followed by *Allium sativum* (97.5 mg GAE/100g) and *Capsicum Annum* L. (92.5 mg GAE/100g).

The results for the antioxidant activity that were expressed as percentage of decrease in the absorbance value of each sample compared with the absorbance of DPPH reference solution, ranged between 10.9 and 18 % following the same pathway as the polyphenolic content did.

Comparing our result with previously published research, we founded similar results for DPPH activity (Bae et al., 2012; Salim et al., 2012, Zhuang et al., 2012).

**Tab. 1.** The content of total phenols and antioxidant activity of *Allium sativum*, *Capsicum Annum* L. and *Piper nigrum* L samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Total phenols (mg GAE/100g)</th>
<th>DPPH%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium sativum</em></td>
<td>97.5±0.707</td>
<td>18.42±0.007</td>
</tr>
<tr>
<td><em>Capsicum Annum</em> L.</td>
<td>92.5±0.707</td>
<td>10.90±0.000</td>
</tr>
<tr>
<td><em>Piper nigrum</em> L.</td>
<td>338±1.414</td>
<td>13.28±0.085</td>
</tr>
</tbody>
</table>

**Tab. 2.** Antimicrobial activity of *Allium sativum*, *Capsicum Annum* L. and *Piper nigrum* L samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dose</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td>50 mg/mL</td>
<td>15.250±0.354</td>
</tr>
<tr>
<td><em>Capsicum Annum</em> L.</td>
<td>50 mg/mL</td>
<td>10.250±0.354</td>
</tr>
<tr>
<td><em>Piper nigrum</em> L.</td>
<td>50 mg/mL</td>
<td>11.750±1.061</td>
</tr>
<tr>
<td>Gentamicine</td>
<td>0.2 mg/mL</td>
<td>26</td>
</tr>
</tbody>
</table>
The antibacterial activity of garlic, red pepper and black pepper extracts were evaluated against Gram-positive (S. aureus) and Gram-negative (E. coli, Salmonella tulphiummirium and P. aeruginosa) strains by measuring the inhibition zone diameter (Table 2). As it can be seen in Table 2, all extracts showed varying degrees of antibacterial activity against of the Gram-positive and Gram-negative bacteria tested. Similar results obtained also Zarai et al., 2013. Spices showed, antibacterial activity against all tested bacteria with zone of inhibition ranged from 8 mm-15.250 mm. The maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (15.250 mm) than Gram negative bacteria Pseudomonas aeruginosa (12.250 mm) and Escherichia coli (8 mm).

CONCLUSION

According to the results obtained, the aromatic spices studied have levels of phenolic constituents that contribute to a high antioxidant activity and may be considered as a good source of natural antioxidants. Our results showed that, when using various in vitro tests of aromatic spices exhibits the highest antioxidant activity. Possesses also an antibacterial activity against all Gram (+) bacteria tested and especially against S. aureus. An antibacterial activity against gram (-) bacteria was also observed. According to these results, selected aromatic spice may be considered as a natural preservative against food-borne pathogens. The information presented in this study can help promote the consumption of spices in fresh or in dry form.

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