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EXPERIMENTAL PAPER

Antioxidant properties of selected culinary spices

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Summary

Introduction: Seasonings added to food enhance its flavor and texture. Some of them can also extend their shelf-lives thanks to the presence of antioxidant compounds. **Objective:** The aim of the study was to investigate twenty eight commercially available spices for the total phenolic contents and antioxidant activity. **Methods:** Total phenols were estimated according to the Folin-Ciocalteu method. Antioxidant activities of the extracts were determined with DPPH assay. **Results:** Our results showed that the most of analysed spices are rich in phenolic compounds and demonstrate good antioxidant activity. The total polyphenol content oscillated around 0.9–155.1 mg GAE/g with the lowest value for sesame and the highest for cinnamon. The DPPH radical scavenging ability expressed as % ranged from 4.1% for sesame to 94.9% for cloves. Moreover, a moderate correlation ($r=0.63$, $p<0.05$) was reported between antioxidant activity and total phenolics, revealing that phenolic compounds are the important antioxidant components in the examined spices. **Conclusion:** The study shows also that a lot of spices can serve as food preservatives and, at the same time, have a beneficial effect on human health.

Key words: *spices, phenolics, antioxidant activity, reactive oxygen species (ROS)*

INTRODUCTION

During the growing process, plants are exposed to various stress factors, both abiotic and biotic. One of the most common plant responses to stressors is an increase in the concentration of reactive oxygen species (ROS). Plant tissues

have an effective defense mechanism against damage caused by ROS, such as the activity of enzymes: superoxide dismutase, catalase and peroxidase, along with non-enzymatic antioxidants: ascorbate, glutathione and terpenoids (carotenoids, tocopherols), especially phenolics, which neutralize ROS. Antioxidants of low molecular weight react directly with the reactive forms of oxygen or indirectly with metabolites of redox reactions, preventing the ROS from formatting [1, 2].

The human body reacts to stress similarly to plants. However, unlike plants, often an imbalance between ROS and endogenous antioxidant levels occurs in human, leading to cell damage [3]. For this reason, it is important to provide ROS in the daily diet, as it plays a key role in elimination of free radicals, which have been linked to cardiac disease [4], cancer [5], neurodegenerative diseases such as Alzheimer's disease [6] and inflammatory diseases [7]. Recently, much attention has been focused on finding compounds present in plants for use as natural antioxidants.

Spice plants are a great source of antioxidants and can be used both in cooking or as food preservatives. Spices used widely in food manufacturing and processing are an alternative to unhealthy, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which contribute to liver damage and carcinogenesis [8, 9]. Seasonings added to food enhance its flavor and texture, can also extend its shelf-life, thanks to the presence of active bacteriostatic and bactericidal compounds and to the inhibition of the oxidation process [10]. Examples of antioxidant compounds found in spices may include eugenol, camphene, carvacrol, piperine, myristic acid, myristicin and ascorbic acid [11].

In this study, 28 commercially available spices were examined with regard to polyphenol content and antioxidant activity. The conclusions suggest that the high radical-scavenging capacity of these spices makes them ideal food preservatives with health-promoting properties.

MATERIAL AND METHODS

Chemicals

Methanol (POCh Gliwice, Poland) was used to prepare the extracts, and Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and sodium carbonate (POCh Gliwice, Poland) to determine the total phenolic content. A 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) assay was used to estimate antioxidant activity. Trolox and gallic acid (Sigma-Aldrich, St. Louis, MO, USA) were used as standards to calculate calibration curves. The water used as reagent for the preparation of solutions was double-distilled.

Plant material

The dried spices were purchased and collected from local supermarkets and chemists in 2014. All the spices used are commercially available, produced by three Polish manufacturers and belong to one of following botanical families: *Apiaceae* (*Umbelliferae*), *Piperaceae*, *Myristicaceae*, *Lamiaceae* (*Labiatae*), *Zingiberaceae*, *Amaryllidaceae*, *Pedaliaceae*, *Solanaceae*, *Myrtaceae*, *Lauraceae*, *Cupressaceae*, *Ranunculaceae*, *Asteraceae*, *Brassicaceae*. The families and scientific names of the tested spices are detailed in table 1.

Preparation of extracts

Spice material was ground into a fine powder (particle size of 700 μm) using a laboratory mill (Bionovo, Legnica, Poland). All the tested samples were weighed (0.250 g) and extracted using 5 ml of 80% methanol for 1 h at room temperature in a shaker (TTS 2, Yellow Line, IKA-Werke GmbH & Co. KG, Staufen, Germany). The homogenates were then centrifuged (type Sigma 2-16P, Polygen, Wrocław, Poland) at 12000 RPM for 30 min. The antioxidant properties of the supernatants obtained were assessed immediately following the method described below. After the analysis, the remaining supernatants were stored at 4°C for further use within 24 h.

Determination of total phenols

The oxidative potential of the tested samples was estimated in terms of the total phenolic content (TP), measured using the Folin-Ciocalteau (FC) method with modifications [12]. To 50 μl of the tested solutions, 3.85 ml of H_2O and 100 μl of undiluted FC reagent were added. The mixture was left for 3 min at a room temperature (RT), after which 1 ml of 10% Na_2CO_3 was added. The control (blank) contained all the reaction reagents except the extract. After 60 min of incubation at 25°C (RT), the exact absorbance was measured against the blank at a wavelength of λ=760 nm. Each measurement was performed in triplicate (n=3) and the average value of absorbance for the sample solution was estimated. The absorbance was measured using a UV–VIS spectrophotometer 8453 (Hewlett Packard/Agilent, USA). The phenolic content was calculated on the basis of a calibration curve (fig. 1) relative to a methanolic solution of gallic acid. The total phenols were described as the amount of gallic acid equivalents (GAE) per gram of spice (mg GAE/g).

Table 1.

Antioxidant capacity and total phenolic content of methanolic extracts from 28 selected spices

Spice	Part of plant	Scientific name	Botanical family	TP [a] [mg GAE/g]	AADPPH [b] [%]	TEAC [c] [μM Trolox/g]
Aniseed	fruit	*Pimpinella anisum*	*Apiaceae* (*Umbelliferae*)	7.4 ± 0.912	44.5 ± 4.715	24.6 ± 0.128
Black pepper	fruit	*Piper nigrum*	*Piperaceae*	6.4 ± 0.795	38.0 ± 0.385	21.0 ± 0.010
Nutmeg	seed	*Myristica fragrans*	*Myristicaceae*	6.3 ± 0.569	29.8 ± 0.116	16.5 ± 0.003
Basil	leaf	*Ocimum basilicum*	*Lamiaceae* (*Labiatae*)	26.5 ± 2.315	94.6 ± 0.753	52.3 ± 0.021
Oregano	leaf	*Origanum vulgare*	*Lamiaceae* (*Labiatae*)	51.3 ± 0.210	94.5 ± 0.227	52.3 ± 0.006
Ginger	rhizome	*Zingiber officinale*	*Zingiberaceae*	14.5 ± 1.569	76.5 ± 1.488	42.3 ± 0.041
Garlic	bulb	*Allium sativum*	*Amaryllidaceae*	2.0 ± 0.015	6.1 ± 0.715	3.4 ± 0.019
Caraway	fruit	*Carum carvi*	*Apiaceae* (*Umbelliferae*)	4.8 ± 0.204	36.2 ± 0.493	20.0 ± 0.013
Sesame seed	seed	*Sesamum indicum*	*Pedaliaceae*	0.9 ± 0.031	4.1 ± 0.187	2.2 ± 0.005
Sweet pepper	fruit	*Capsicum annuum*	*Solanaceae*	17.1 ± 2.057	70.4 ± 0.419	39.0 ± 0.011
Red pepper	fruit	*Capsicum annuum*	*Solanaceae*	19.1 ± 1.468	87.0 ± 0.653	48.2 ± 0.018
Clove	flower buds	*Syzygium aromaticum*	*Myrtaceae*	154.1 ± 6.741	94.9 ± 0.226	52.5 ± 0.006
Cinnamon	bark	*Cinnamomum zeylanicum*	*Lauraceae*	155.1 ± 0.961	92.5 ± 0.303	51.2 ± 0.008
Juniper	fruit	*Juniperus communis*	*Cupressaceae*	10.4 ± 0.661	68.8 ± 0.294	38.1 ± 0.008
Lovage	leaf	*Levisticum officinale*	*Apiaceae* (*Umbelliferae*)	17.8 ± 0.356	74.8 ± 3.218	41.4 ± 0.089
Black seed	seed	*Nigella sativa*	*Ranunculaceae*	2.7 ± 0.059	12.2 ± 0.405	6.8 ± 0.011
Pimento	fruit	*Pimenta dioica*	*Myrtaceae*	2.5 ± 0.255	10.9 ± 0.877	6.0 ± 0.024
Cardamom	seed	*Elettaria cardamomum*	*Zingiberaceae*	2.2 ± 0.010	11.1 ± 1.316	6.1 ± 0.036
Turmeric	rhizome	*Curcuma longa*	*Zingiberaceae*	42.9 ± 6.093	89.8 ± 0.227	49.7 ± 0.006

Spice	Part of plant	Scientific name	Botanical family	TP [a] [mg GAE/g]	AADPPH [b] [%]	TEAC [c] [μM Trolox/g]
Onion	bulb	*Allium cepa*	*Amaryllidaceae*	3.2 ± 0.267	5.6 ± 0.101	3.1 ± 0.003
Savory	leaf and branch	*Satureja hortensis*	*Lamiaceae* (*Labiatae*)	48.4 ± 0.396	93.5 ± 0.006	51.8 ± 0.005
Tarragon	leaf and branch	*Artemisia dracunculus*	*Asteraceae*	41.2 ± 3.841	91.3 ± 0.155	50.5 ± 0.004
Thyme	leaf and branch	*Thymus vulgaris*	*Lamiaceae* (*Labiatae*)	71.7 ± 3.522	93.1 ± 0.308	51.5 ± 0.009
Marjoram	leaf and branch	*Origanum majorana*	*Lamiaceae* (*Labiatae*)	43.6 ± 0.779	94.2 ± 4.089	52.1 ± 0.113
Laurel	leaf	*Laurus nobilis*	*Lauraceae*	44.4 ± 2.522	93.8 ± 4.351	51.9 ± 0.120
Parsley	leaf	*Petroselinum crispum*	*Apiaceae* (*Umbelliferae*)	13.6 ± 0.166	30.4 ± 0.484	16.8 ± 0.013
Mustard	seed	*Sinapis alba*	*Brassicaceae*	32.5 ± 0.517	36.8 ± 2.342	20.4 ± 0.065
Chilli pepper	fruit	*Capsicum frutescens*	*Solanaceae*	7.1 ± 0.162	22.1 ± 1.180	12.2 ± 0.033

Note: Value are means ± standard deviation (n=3).

[a] TP is total phenolic content expressed as milligrams of gallic acid (GAE) equivalents per gram of sample.

[b] AADPPH stands for antioxidant activity against DPPPH free radical expressed as inhibition percentage.

[c] TEAC is trolox equivalent antioxidant capacity expressed as micromoles of Trolox equivalents per gram of sample.

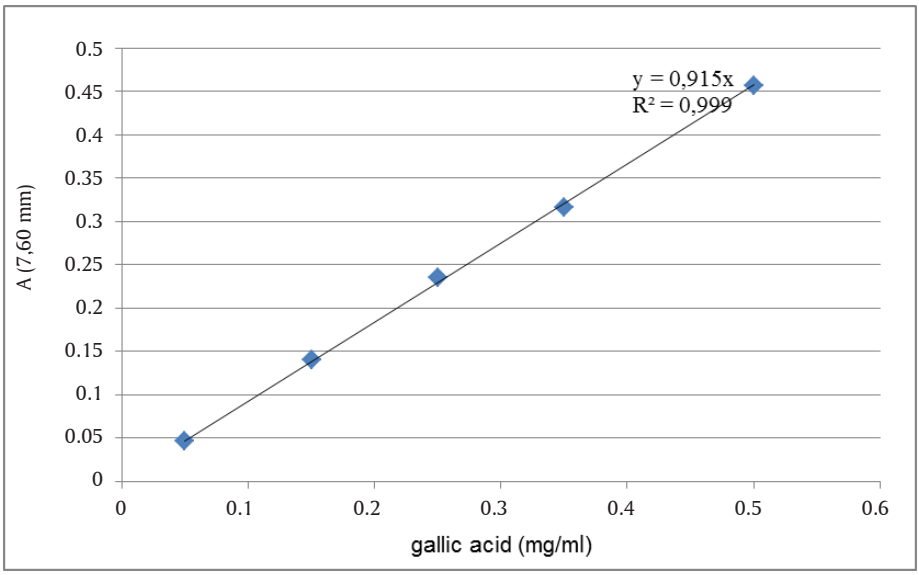

Figure 1.
Calibration curve of absorbance versus concentration of gallic acid [mg/ml]

Determination of antioxidant activity using DPPH reagent

Antioxidant activity was determined using the modified method developed by Brand-Williams *et al.* [13] using the DPPH radical. 0.5 mM methanolic DPPH solution was prepared by dissolving 19.71 mg of DPPH (M=394.32 g/mol) in 100 ml of 80% methanol. The resulting solution was diluted in order to obtain an absorbance value of approximately 0.9 at a wavelength of λ=517 nm. The spectrophotometer was calibrated using 80% methanol (blank). The absorbance of the DPPH radical solution (A_0, control) was measured by adding 3 ml of DPPH solution to 40 μl of 80% methanol. The tested samples contained 3 ml of DPPH solution and 40 μl of the tested extract. Thirty minutes after the initiation of the reaction, the absorbance was compared with the blank reagent at a wavelength of λ=517 nm. Each measurement was conducted in triplicate and the average absorbance of the tested solution was calculated. The results were presented in terms of the percentage of radical scavenging activity according to the following equation:

Inhibition % = 100 (A_0–A)/A_0, where:
A- average absorbance of the sample
A_0- average absorbance of control (DPPH solution).

The antioxidant content was plotted on a calibration curve (fig. 2) against a methanolic solution of Trolox. The total antioxidant content was measured in terms of its Trolox equivalent antioxidant capacity (TEAC), expressed as μM Trolox per gram of spice (μM Trolox/g).

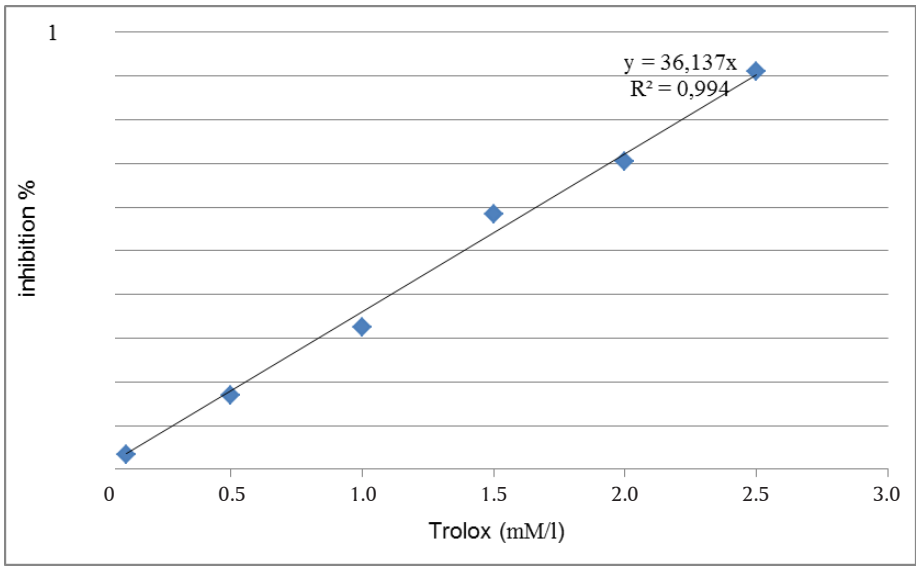

Figure 2.
Calibration curve of % reduction of DPPH radical versus concentration of Trolox [mM/]

Statistical analysis

The results were calculated as the mean ± standard deviation (SD) from tests in triplicate. Correlation analysis was performed using the Pearson's correlation coefficient (r). Both calculations were performed with Microsoft Excel 2007.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

Taking into account the results obtained from the examined spices (fig. 3) it can be concluded that the highest phenol content was in cinnamon and cloves, which contained 155.1 and 154.1 mg GAE/g, respectively. This value was several times higher than that of the other samples. The lowest polyphenol content was found in sesame, at 0.9 mg GAE/g. Some samples had similar total polyphenol contents, starting from garlic (with less than 10 mg GAE/g) to, in order, cardamom < pimento < black seed < onion < caraway < nutmeg < black pepper < chilli pepper < aniseed < juniper. The polyphenol content in parsley < ginger < sweet pepper < lovage < red pepper was within the range 13.6–19.1 mg GAE/g. Basil < mustard < tarragon < turmeric < marjoram < laurel < savory < oregano < thyme showed the range from 26.5 to 71.7 mg GAE/g.

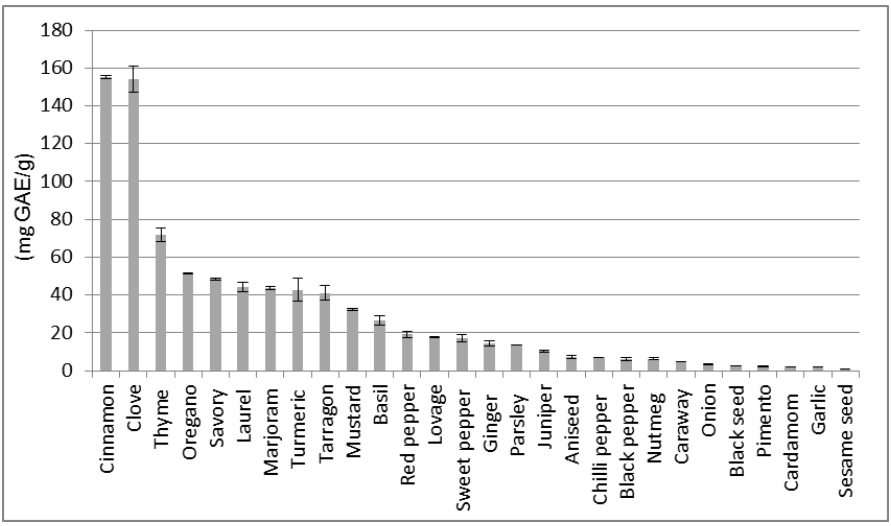

Figure 3.
The content of total polyphenols in the examined spices

The DPPH radical scavenging ability expressed as a percentage varied and ranged from 4.1% for sesame to 94.9% for cloves (fig. 4). Cloves showed the greatest ability to scavenge free radicals, with an antioxidant activity of 94.9%.

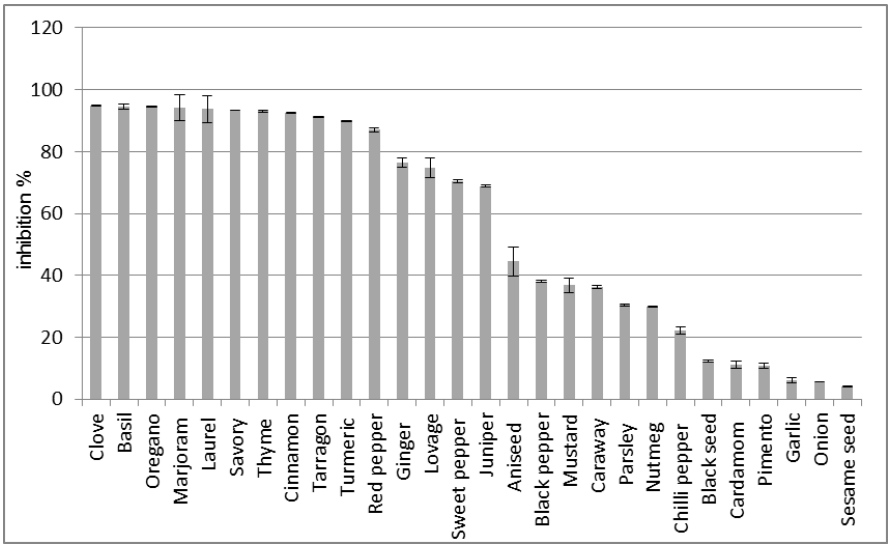

Figure 4.
Antioxidant activity expressed as % scavenging DPPH free radical by examined spices

A similar level was reported, in decreasing order, for basil > oregano > marjoram > laurel > savory > thyme > cinnamon > tarragon > turmeric. The

lowest value of antioxidant activity was found in sesame (4%), followed by onion (5.6%), and garlic (6%). The TEAC values for these spices ranged from 2.2 μmol to 52.5 μmol per 1 g of spice. Examination of the methanolic extracts using the Pearson correlation coefficient revealed a moderate correlation ($r=0.63$) between the polyphenol content and antioxidant capacity measured against DPPH radicals. These results show that spices can behave as free radical scavengers to various degrees and have different polyphenol contents. This finding supports previous studies of spices in which similar results were obtained. However, discrepancies exist with other research, which will be discussed hereafter.

The antioxidant activity of spices can be influenced by many factors. The most significant are those related to the botanical origin and antioxidant content of the spices. However, the literature suggests that the amount of antioxidants in the sample also depends on the type of solvent used for their extraction [14]. Different solvents with various polarities have been used in studies on the antioxidant capacity of spices, including acetone, toluene, ether, methanol, ethanol, ethyl acetate and water. This may explain the different results obtained from the same spices [15]. The polyphenol content in 70% acetone extracts from dried spices expressed as g GAE/100 g of spices in study of Wojda [10] after conversion to mg GAE/g was nearly twice lower (26.7 mg/g) than the values we found for oregano (51.3 mg/g), but similar for garlic (2.2 mg/g). Their granulated garlic acetone extracts, however, showed greater inhibition (14.09%) than our methanol (6.1%) extracts, while in the case of oregano, the opposite effect was observed: 38.33% inhibition for acetone, 94.5% for methanol extracts.

The total polyphenol contents estimated by other authors are also difficult to compare with those found in our research. This is due not only to the different extraction methods, but also to the calculation methods used. Some authors report polyphenol content as mass (mg) per weight of dry extract and display information on the extraction yield as mg of extract from 1 g of dry plant material [16]. Other sources calculate polyphenol content as a % of dry weight [17]. Still, others measure polyphenol content as mg/100 g fresh weight [18]. Different standards are also used to draw the calibration curve of polyphenol content, which is usually expressed as the gallic acid equivalent, but which is also sometimes given as the equivalent of catechin [16], catechol [19], pyrogallol or caffeic acid [17].

These difficulties in the comparison of the results are caused by the fact that the phenolic compound content varies, even within the same spice plant. This can be due to many factors such as the place of production and environmental stressors [20]. It also depends on the part of the plant chosen for test, time of harvest and methods used to determine phenolic compound content [21]. Measurements of total amount of polyphenols using the Folin-Ciocalteu method may, furthermore, be distorted by interference of other compounds present in plants [20].

In our study, high levels of polyphenols, ranging from 26.5 to 71.7 mg GAE/g, were found in all analyzed spices belonging to the family *Lamiaceae* (basil, oregano,

savory, marjoram, thyme). Due to the presence of phenolic compounds with high biological activity, plants from this family are used in medicine, particularly to stimulate digestion and appetite, but also as diuretic, antispasmodic, antibacterial and expectorant remedies [17]. According to a study by Hossain *et al.* [22], the major phenolic compounds present in plants of this family are hydroxycinnamic acids (caffeic, chlorogenic, *p*-coumaric, rosmarinic and ferulic), flavonoids and hydroxybenzoic acids, which exist mainly as esters and glycosides [17]. Of these compounds, rosmarinic acid exhibits the highest scavenging activity, due to the fact that its molecule contains four hydroxyl groups [23]. Oregano and thyme have been identified as having high rosmarinic acid content [24].

Some studies [16, 20, 24] have shown a high correlation between the content of polyphenols and antioxidant activity, while others [18, 19, 25] describe a weak linear correlation or even a lack of correlation [26]. Our results reveal a moderate correlation of $r=0.63$ ($p<0.05$), which indicates that the contents of phenolics in the tested plants explain nearly 40% of the antioxidant activity variation. Phenolic acids and flavonoids play an important role in scavenging free radicals [26]. Residual antioxidant activity (about 60%) may result from active ingredients present in spices, such as essential oils, carotenoids, vitamins and other glycosides [19].

The total polyphenol content in 0.1% aqueous marjoram extracts of various origin determined by Newerli-Guz [27] ranged from 26.66 to 54.64 mg GAE/g and their DPPH quenching ability from 87.95% to 89.19%. The results obtained from our study for marjoram fall within this range (43.6 mg GAE/g). However, the antioxidant activity is slightly higher (94.2%), which may result from the use of methanol to extract the antioxidants from the sample. In the work by Newerli-Guz [28], the total polyphenol content for all tested samples of 1% aqueous extract of black pepper was 13.45 mg GAE/g. In our study, the result we obtained was more than 50% lower (6.4 mg GAE/g). The DPPH scavenging activity in all tested samples of black pepper was high and reached a value of about 80%, in comparison to that from our study which amounts only to 38%. Newerli-Guz also shows that the ability of black pepper to scavenge free radicals depends on the intensity and duration of the ionizing radiation to which the samples are subjected. Additional factors which could have an impact are the length of extraction time, the specific reagent used in the assay (the type of applied research protocol), together with other factors such as genetic and environmental diversity (climate, temperature, fertility, diseases and exposure to pests) [29].

Similarly to our study, Kim *et al.* [16] found cloves to have the highest antioxidant activity. However, there is a difference in the inhibition these authors found for this spice (84.22%), lower than the value found in our study (94.9%). Other studies had also suggested that cloves have very strong antioxidant activity and high levels of phenols [16, 21, 30]. Our results confirm that the extract from the buds of cloves is the most powerful phenolic antioxidant and has the strongest free-radical scavenging activity out of the 28 spices analyzed.

The antioxidant activities of other spices such as thyme (70.79%), savory (53.51%), oregano (45.43%), basil (39.63%), marjoram (30.22%) and turmeric (24.43%) were also lower in the study by Kim *et al.* [16] than in our results, which shows values for these spices as 93.1%, 93.5%, 94.5%, 94.6%, 94.2% and 89.8%, respectively. These differences may be due to the fact that Kim *et al.* [16] used aqueous extracts, whereas we worked with methanol extracts, which was found to be a more efficient solvent for antioxidants extraction. The antioxidant activities of other analysed spice plants from the *Lamiaceae* family were of a similar level, or at lower levels for nutmeg (20.94%) and caraway (30.67%). The polyphenol content (80%) reported by Shan *et al.* [21] for methanolic extracts of spices expressed in g/100 g dry weight are similar to those found in this study.

CONCLUSIONS

This study has confirmed that spices can be a potential source of natural antioxidant substances, focusing on a group including cloves, marjoram, savory, laurel, cinnamon, tarragon, turmeric and spice plants from the *Lamiaceae* family. The high radical-scavenging capacity of these spices makes them ideal food preservatives with health-promoting properties.

Conflict of interest: Authors declare no conflict of interest.

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ANTYOKSYDACYJNE WŁAŚCIWOŚCI WYBRANYCH PRZYPRAW

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Streszczenie

Wstęp: Przyprawy dodawane do żywności wzbogacają jej walory smakowe i zapachowe, niektóre z nich mogą także wydłużać jej czas przechowywania dzięki obecności związków o aktywności przeciwutleniającej. **Cel:** Celem pracy było zbadanie 28 przypraw dostępnych na rynku pod kątem całkowitej zawartości polifenoli i aktywności przeciwutleniającej. **Metody:** Do oceny całkowitej zawartości związków fenolowych zastosowano metodę Folina-Ciocalteau. Aktywność antyoksydacyjną ekstraktów oznaczono metodą z użyciem rodnika DPPH. **Wyniki:** Otrzymane wyniki wykazują, że większość analizowanych przypraw jest bogata w związki fenolowe i posiada wysoką aktywność antyoksydacyjną. Całkowita zawartość polifenoli w przyprawach oscylowała od 0,9 do 155,1 mg GAE/g z najniższą wartością odnotowaną dla sezamu i najwyższą dla cynamonu. Zdolność do wygaszania rodnika DPPH wyrażona w procentach była zróżnicowana i wahała się w zakresie od 4,1% dla sezamu do 94,9% dla goździków. Odnotowano umiarkowaną korelację ($r=0,63$, $p<0,05$) pomiędzy aktywnością antyoksydacyjną i zawartością polifenoli ogółem, co dowodzi, że związki fenolowe są ważnymi, antyoksydacyjnymi składnikami w badanych przyprawach. **Wnioski:** Prowadzone badania wykazują, że wiele przypraw może służyć jako naturalne konserwanty żywności i jednocześnie ma korzystny wpływ na nasze zdrowie.

Słowa kluczowe: *przyprawy, fenole, aktywność przeciwutleniająca, reaktywne formy tlenu ROS*