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PRELIMINARY STUDY REGARDING THE BIOLOGIC ACTIVITY OF SPICES

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Abstract

Cinnamon, cloves, and turmeric have long been valued as aromatics and components in oils and fragrances, and have been used as spices and remedies since ancient times. Ground spice is crucial in the food business for seasoning baked goods such as cakes, buns, biscuits, cookies, steaming puddings, pies, sweets, chewing gum, and desserts. However, the food business can benefit from the antioxidant activity of essential oils, for functional food production, with potential health claims. The paper aimed to evaluate the biologic activity (total phenolic content, the antioxidant activity and the antimicrobial effect) for the grounded and oil form of cinnamon, cloves, and turmeric. Among the evaluated spices, the grounded clove indicates the highest antioxidant activity (p<0.05), when compared with grounded cinnamon and turmeric. The value of the total phenolic content present in the grounded cloves oils was the highest (284.42 ± 32.98 mg GAE/100 mL), when compared with cinnamon oil (100.43 ± 9.85 mg GAE/100 mL), and turmeric oil (166.50 ± 12.76 mg GAE/100 mL). The biologic activity of the spices suggests that in addition to imparting flavour to the food, they possess high potential as health promoters by their antioxidant effect, phenolic content and might be suitable for antimicrobial activity.

Key words: *evolution, milk production, NW Region, Romania, trends.*

INTRODUCTION

Free radicals are formed both as a result of natural processes that take place in the body (Chaudhary et al., 2023), and under the action of external factors, like oxidative stress (Sharma & Mehdi, 2023), thermal stress of dietary fats (Mircea & Mititelu, 2023), unhealthy diet (Mustafa, 2023), sedentary lifestyle, cigarette smoke (Seo et al., 2023), pollution, drugs, radiation (Liu et al., 2023).

The body's cells (DNA, cell membrane) can be affected by their existence, because they are characterized by increased reactivity.

Although the human body has the ability to produce antioxidants, with a role in neutralizing free radicals by giving up spare electrons, in some cases the number of free radicals formed can be greater than the antioxidants that can neutralize them (Parcheta et al., 2021).

In this context, an intake of antioxidants (food, spices) is essential. Antioxidants present in food determine numerous benefits for the food product: they slow down the formation of oxidation production with toxic potential, control the development of the rancidity process, protect lipids and oils against oxidative food degradation (Akbari et al., 2022), contribute to the preservation of the nutritional characteristics of food and the extension of the shelf life (Munekata et al., 2020).

Turmeric (curcuma) is a herbaceous plant that belongs to the genus *Curcuma longa*, the ginger family, and is recognized for its powerful anti-inflammatory and antioxidant properties (Srivastava et al., 2022).

The main active ingredient, curcumin, can be extracted from the turmeric rhizome and is found at 2.5 to 4% in turmeric powder (Van Nong et al., 2016).

To benefit from the benefits of turmeric, it is recommended to take supplements in which curcumin is bound to phosphatidylcholine (Began et al., 1999), for better absorption and bioavailability.

Studies carried out on the properties of curcumin on the body have shown that it also contributes to the stimulation of the neurotrophic factor derived from the brain (Hosseinzadeh et al., 2013; Tizabi et al., 2014).

Cinnamon (*Cinnamomum verum*) is one of the oldest spices, being well-known for its characteristic, intense aroma. The high content in polyphenols, as well as the characteristic active principles (cinnamaldehydes, alcohol and cinnamyl acetate) determine antioxidant properties (Al-Mijalli et al., 2023), with a role in protecting cells against degradation. Cinnamon essential oil contains 90% cinnamaldehyde, which works to improve blood circulation, regulate blood pressure, and reduce platelet viscosity. Among its many benefits, cinnamon is a neurological tonic, with effects on stimulating brain activity, improving attention and memory (Tabassum et al., 2012). According to some studies, satisfactory results can also be obtained in the regulation of blood glucose, cholesterol and triglyceride levels (Ayati et al., 2021). Cinnamon can also be administered to combat bacterial and fungal infections (Ebani & Mancianti, 2020), being effective in inhibiting the development of some bacteria - *Listeria* sp. (Somrani et al., 2020), and *Salmonella* sp.

Cloves (dried buds of the flowers of the clove tree - *Syzygium aromaticum*) belong to the category of the most valuable spices and have been used since ancient times, both for flavouring and preserving food, and for therapeutic purposes. The rich content of volatile compounds gives cloves their characteristic aroma, and eugenol, which is the majority component (70-90%) in clove oil determines the anti-inflammatory properties for which it is recognized, especially in toothaches. Also, clove oil has antibacterial and antifungal effects, being effective in treating infections caused by Candida. The high antioxidant content of clove essential oil helps to significantly reduce oxidative stress.

This study aims to investigate the biologic activity (total phenolic content, the antioxidant activity and the antimicrobial effect) for the grounded and oil form of cinnamon, cloves, and turmeric.

MATERIALS AND METHODS

Materials

Three beneficial bivalent (food and medicinal) spices (cinnamon, cloves and turmeric) powders and oils were represented by commercial formula products (powder and oils), purchased from Romanian trade markets. The vegetal materials were scavenged for total phenolic content and total antioxidant activity, and *in vitro* determination for antimicrobial effects.

Analytical grade reagents and standards were represented by: Folin-Ciocalteu (Merck, Germany), methanol, sodium carbonate, gallic acid, and 2,2-diphenyl-1-1picrylhydrazyl, purchased all from Sigma-Aldirch, Missouri, USA.

Total phenolic content

For evaluating the total phenolic content, the Folin-Ciocalteu method was adapted for laboratory conditions, using a spectrophotometer (PG instruments® T60, UV-Vis spectrometer, Tokyo, Japan), for measuring the absorption at fixed wavelength, 765 nm. Individual spices samples were weighed (5 ± 0.57 g) an analytical balance (KERN ABJ 220-4M), and each oil sample was measured (1 mL) using the automatic pipette. The samples (powders and oils) were transferred to balloon flask 100 ml, and vortexed for 5 minutes (IKA®Vortex 3, Werke GmbH & Co. KG., Germany) with 70% methanolic solution, preheated at 70°C (30:70, ultrapure water: methanol). The incubation time for the samples was 24 h at 70°C. The samples mixture was separated using a centrifuge (Boeco C28-A, Germany), at 5000 rpm, 10 min. The resulted spices supernatants were collected (1 mL) and mixed with 5 mL Folin-Ciocalteu (1:1, F-C: ultrapure water), 1 mL ultrapure water and 4 mL Na_2CO_3. The samples stand for 30 minutes at room temperature 20°C, in dark conditions.

Blank sample was prepared in a similar way to the standard curve without adding the gallic acid. The calibration curve, having different gallic acid concentrations (10, 20, 30, 40, and 50 μg/mL) was done in order to calculate the total phenolic content, expressed as milligrams of gallic acid equivalents per g of extract (mg GAE/g).

Antioxidant activity

By using 2,2-diphenyl-1-1picrylhydrazyl (DPPH), the antioxidant activity was measured, for cinnamon, cloves, and turmeric. The powders extracts were individually prepared weighing 5 ± 0.65 g and mixing with 20 mL 80% methanolic aqueous solution, and incubated at 40°C, during 30 minutes. After separation (5000 rpm, 10 minutes), the supernatant was collected, and the volume of 500 µL of extracted sample was homogenised with 500 µL 80% methanolic aqueous solution, and 5 mL DPPH, and left to rest in dark conditions, during 30 minutes. By using the PG instruments® T60, UV-Vis's spectrometer (Tokyo, Japan), the absorbance of the test samples was measured at 517 nm fixed wavelength, and antioxidant activity results were represented as a percentage:

$$\text{Antioxidant effect \%} = \frac{X_c - X_s}{X_c}\ x\ 100,$$

X_c - Absorbance of control;
X_s - Absorbance of tested sample.

Antimicrobial effect

The disk infusion assay (Figure 1) was employed to determine the antimicrobial activity of spices (n = 3, powder). Different individual concentrations were prepared as soaked disks, in water (concentration ranged 50, 100, and 150 mg/dL) and alcohol 96% ethanol (concentration ranged 50, 100, and 150 mg/dL). The control was represented by antibiotic active substances specific action (Amoxicillin, Sulfamethoxazole, Streptomycin, purchased from Oxoid™ LTD, Ontario, Canada). Individual 1 cm^3 x 10^9CFU (0.5 McFarland standard) of *Enterococcus faecium* ATCC 51559, *Escherichia coli* ATCC 8739, and *Staphylococcus aureus* subsp. *aureus* ATCC 25923 (ATCC, Virginia, USA), were inoculated by flooding technique, in 9 mm diameter Petri dish, on TSA nutritive media (Tryptic Soy Agar, Merk), incubated for 24 h (LLG-uniINCU 28 COOL, Precisa, Germany), at 37°C. For evaluation, the presence of inhibition was taken into consideration, inclusively the diameter of the inhibition area, in mm.

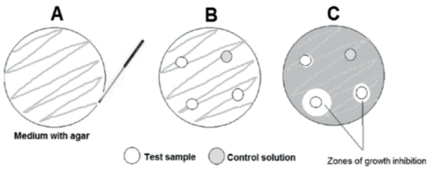

Figure 1. Graphic representation of evaluation of antibacterial activity, by using the disk diffusion test: (A) Petri dish containing culture medium on agar nutritive media; (B) Paper disks soaked with control solution or different concentrations placement; (C) Results after incubation bacterial colonies and the zones of growth/ inhibition observed in the sample exerted of the antibacterial effect of tested samples (after Pavia et al., 2013)

Statistical analyses

Data were analysed employing the ANOVA procedure with SPSS software (version 20 Inc. Chicago, IL, USA). When comparing group means, Post hoc Tukey's test was carried out to evaluate the significant differences for the measured parameters. Statistical differences were considered significant at a level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Total phenolic content

The total phenolic content in plants exhibit high redox characteristics, participating in the antioxidant activity (Aryal et al., 2019). Hydroxyl groups within plant extracts aid in free radical scavenging (Lalhminghlui & Jagetia, 2018). The results for the total phenolic content are displayed in the Table 1.

Table 1. Total phenolic content of spices

Sample	Mean*	SD	CV
Cinnamon powder[1]	17.65	2.44	13.82
Cloves powder[1]	299.54[a]	12.34	4.11
Turmeric powder[1]	198.28[b]	27.89	14.07
Cinnamon oil[2]	100.43	9.85	9.81
Cloves oil[2]	284.42[a]	32.98	11.59
Turmeric oil[2]	166.50[b]	12.76	7.67

*Mean of n = 3 tested samples; the results were expressed as equivalent of gallic acid, in mg /100 g product; [a, b]different superscript highlights the statistical significances at the level of interest $p<0.05$; [1]mg/100 g product; [2]mg/100 mL product.

The 70% methanol is an efficient solvent for extracting antioxidant compounds, due to its polarity and the capacity to solubilize and recover phenolics from plant matrices, according to previous literature reviews (Li et al., 2008; Sultana et al., 2014; Zhang et al., 2023). All our tested spices, in oil and powder form expressed total phenolic content. Results on this study indicate that cloves oils and powder have a significant greater amount of total phenolic content, 284.42 ± 32.98 mg GAE/100 mL, respectively 299.54 ± 12.34 mg GAE/100 g, when compared with cinnamon and turmeric powder and oils. Turmeric powder and oil had significant amounts of total phenols, 198.28 ± 27.89 mg GAE/100 g, and 166.50 ± 12.76 mg GAE/100 mL, when compared with the cinnamon powder and oil formula. Similar to our results (Ereifej et al., 2016; Hossain et al., 2023; Sellami et al., 2013) found that methanolic extraction of cloves had highest amounts of total phenolic content.

Antioxidant activity

The antioxidant activity of spice's (powder and oils) measured by the DPPH assay followed the same trend with the total phenolic content assay (Figure 2).

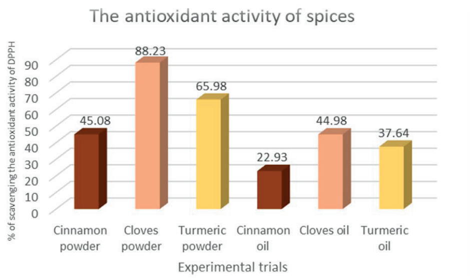

Figure 2. The total antioxidant activity of spices

As previously studied (Grigore et al., 2023; Saranya et al., 2017), the antioxidant capacity was highly dependent on the constituent composition within the extractor capacities. However, there are other assays for scavenging the antioxidant capacity of vegetal matrices, such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (De-Montijo-Prieto & Razola, 2021). For evaluating the ability of a molecule to scavenge or eliminate the free radicals, we consider that DPPH stands out due to the fact that some chemical compounds (polyphenols, carotenoids and pigments) may interfere within the ABTS assay due to their absorbance at the wavelength used for measurement (Yehmed et al., 2023).

The highest antioxidant activity was obtained when testing the cloves powder and cloves oil, 88.23%, and 44.98%, when compared with turmeric and cinnamon (powders and oils). Cloves demonstrate significant antioxidant activity attributed to their substantial levels of phenolic compounds (Frohlich et al., 2023), flavonoids (Kiki, 2023), and essential oils (Manzoor et al., 2023). The presence of these bioactive constituents enables cloves to effectively neutralize free radicals (Aldabaan et al., 2024), thereby mitigating oxidative stress and potentially mitigating the incidence of developing chronic diseases (Rani et al., 2023) linked to free radical-induced damage. Introducing cloves into dietary regimens or employing clove extracts may serve as a strategy to bolster the body's antioxidant defenses and counteract oxidative stress-related health concerns.

In accordance to our results, others studies regarding the antioxidant activity showed similarities regarding the cloves (Frohlich et al., 2023; Kiki, 2023), turmeric (Ballester et al., 2023; Tüzün & Saraç, 2023), and cinnamon (Pagliari et al., 2023).

Antimicrobial effect

The antimicrobial activity was evaluated by employing the principal disk diffusion method, for water and alcoholic extracts of spices (Table 2).

Table 2. Antimicrobial effect of spices[1]

Sample (mg/dL)	*E. faecium*		*E. coli*		*S. aureus*	
	H_2O	C_2H_5OH	H_2O	C_2H_5OH	H_2O	C_2H_5OH
Cinnamon 50	1.74	2.33	0.41	1.52	0.34	1.74
Cinnamon 100	2.55	3.62	1.08	4.75	0.71	8.87
Cinnamon 150	3.98	8.22	2.41	6.23	2.31	10.33
Cloves 50	2.06	3.76	1.18	3.21	4.44	12.89
Cloves 100	2.73	5.53	3.44	8.79	6.44	13.39
Cloves 150	4.12	8.77	5.37	12.11	8.56	15.77
Turmeric 50	1.96	2.60	1.03	2.74	0.72	5.98
Turmeric 100	3.42	6.01	2.84	6.21	2.78	8.21
Turmeric 150	5.29	9.02	6.09	8.76	5.61	8.99
Control	Amoxicilin		Sulfamethoxazole		Streptomycin	
10 µg/mL	0.00		0.00		0.00	

[1]The results are expressed as means of the measured diameters (n = 3, mm) of the inhibition zones formed.

The control was represented by different commercial antibiotic active substances, well known for their inhibitory effect spectrum (Amoxicillin, Sulfamethoxazole, Streptomycin) in the concentration equal to 10 µg/mL. The control plates shown inhibition of cellular development, from the start of the experimental trials.

The largest diameter of the spice's inhibition zone was shown at a concentration of 150 mg/dL of cloves alcoholic extract, measuring 15.77 mm, which was tested against *Staphylococcus aureus* ATCC 25923, and the following measuring 13.39 mm, in the same biologic conditions, the concentration equal to 100 mg/dL cloves alcoholic extract. By increasing the concentration of cloves extract is directly proportional accompanied by the diameter of the inhibition zone, for this small range of concentrations, which is increasingly various compounds.

Same trends were observed for turmeric, who had best results inhibiting the *Enterococcus faecium* strain ATCC 51559, average value of the measuring diameters was equal to 9.02 mm, when concentrated at 150 mg/dL, in alcoholic extraction. Moreover, when comparing the antimicrobial activity of the current three spices, we could highlight the fact that, the smallest activity concerning the antimicrobial effect, was observed for the cinnamon water extract, having the smallest mean values measured for the concentration 50 mg/dL, against the *Escherichia coli* strain ATCC 8739.

Nevertheless, similar results were published (Parisa et al., 2019; Rayess et al., 2023), when evaluating spices for their antimicrobial effects.

CONCLUSIONS

The tested spice that showed the highest antioxidant activity was represented by the cloves. The food formulates obtained oils from aromatic plants showed high antioxidant activity and sufficient amounts of total phenols. The strong antimicrobial activity of cloves powder and the large spectrum they have shown to provide evidence that they might represent a potential solution prolonging the shelf life of food products, developing functional foods, and for protecting plants and crops, and of course they can be an important raw material for the bio-therapeutic cosmetics, for anti-aging and antioxidant effects. Future researches are needed in order to evaluate the mechanisms throughout the antioxidant and anti-aging effects and the interactions between the nutritional effects of the antioxidant plant materials.

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