Evaluation of Antioxidant Activity and Total Phenols Content in Selected Spices

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The total phenols contents and antioxidant activities of alcoholic extractive solutions of Ocimum basilicum (basil), Thymus vulgaris (thyme), Mentha piperita (mint), Rosmarinus officinalis (rosemary), Sativa officinalis (sage), Artemisia dracunculus (tarragon) and Coriandrum sativum (coriander) were examined using Folin-Ciocalteu method and ACL method, respectively. Extractive alcoholic solutions of above mentioned seven spices were analyzed during a period of five months in order to determine the optimal extraction time when the polyphenolic content was at maximum. The highest total phenolic contents were registered for rosemary (608.37 mg GAE/100 g d.w.), sage (530.66 mg GAE/100g d.w.) and mint (511.87 mg GAE/100g d.w.) In general the optimal extraction time of the total phenolic content was reached after 3 months of maceration, except thyme, tarragon (after two months) and coriander (after one month). The antioxidant capacity ranged between 1410.1 nmols Trolox/g d.w. for rosemary and 4.2560 nmols Trolox/g d.w. for tarragon.

Keywords: spice, total phenols, extraction time, antioxidant activity, Folin-Ciocalteu, ACL

Aromatic plants like basil, thyme, rosemary, sage, tarragon, coriander are excellent sources of secondary metabolites, in particularly phenolic compounds that are associated with antioxidative and antimicrobial action in all biological systems [1].

Generally, industrial foods are developed to supply the requirements of consumers in relation to taste, appearance, market value, and practicality, to prepare/consume.

The use of spice in foods has been known since antiquity to improve the flavour or to possess antibacterial and antifungal properties. Spices represent natural sources of polyphenolic compounds [2-4].

Many epidemiological studies have shown that antioxidants from food have significant properties for the prevention of several pathologies, including various types of cancer, heart disease, neurological diseases and other disorders linked to aging [5-8].

A main cause of food deterioration is the lipid peroxidation that consists on formation of reactive oxygen species and free radicals which can be associated with carcinogenesis, mutagenesis, inflammation, DNA changes, aging and cardiovascular diseases [9-13].

Synthetic antioxidants, such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and *tert*-butyl hydroquinone (TBHQ), are frequently used in food industry because they are effective and less expensive than natural antioxidants [14-21].

The spices' capacity to improve food flavour and also the high concentration of polyphenolic compounds recommends them as the best choice in replacing synthetic antioxidants in food systems and offer additional health benefits [22].

The aim of present study was to evaluate the antioxidant properties of spices extractive solutions and to determine the optimal extraction time to obtain the highest total phenols concentration.

Experimental part

Plant materials

Seven spices: *Ocimum basilicum* (basil), *Thymus serpyllum* (thyme), *Mentha piperita* (mint), *Rosmarinus officinalis* (rosemary), *Sativa officinalis* (sage), *Artemisia dracunculus* (tarragon) and *Coriandrum sativum* (coriander) have been picked up in June 2015 from Dobrogea County, Romania (gardens or field). Plant materials consisted of seeds (coriander) and aerial parts (basil, thyme, mint, rosemary, sage, tarragon).

Chemicals

All used reagents were of analytical reagent grade. Gallic acid was purchased from Fluka (Buchs, Switzerland) and Folin – Ciocalteu reagent from Merck (Darmstadt, Germany). Gallic acid (standard phenolic compound) 1×10^{-2} mol×L^{-1} was prepared by dissolving 0.1881g of gallic acid in 100 mL of ethanol. Folin – Ciocalteu reagent was diluted with distilled water 1:10 (V:V).

Apparatus

Spectrometric measurements were carried out using a UV-Vis JASCO V550 scanning spectrophotometer. The antioxidant capacity of lipid soluble substances determination has been performed with Photochem® instrument with ACL kit (Analytic Jena AG, Germany).

Sample extracts

Extractions were achieved by maceration of 10 g of dried plant product in 100 mL ethanol (p.a, Merck) at room temperature. The mixtures were strongly shaken three times every day. In day 3, 7, 14 and 30 of the first month and on day 30 of the next four months, 5 mL alcoholic extract, previously filtered, was collected each time and used to plot absorption spectra in the range of 500-900 nm and to measure the total phenols content.

Total phenols (TPC)

The total phenols were estimated according to the Folin-Ciocalteu method [23-24]

To 5 mL extractive solution were added 1 mL of Folin-Ciocalteu-reagent 1:10 (V:V) and 1 mL of 20% (w/v) aqueous Na_2CO_3; after 10 min the volume was made up to 50 mL with distilled water. After another 30 minof incubation at 25°C the absorbance was measured at 675 nm; the

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total phenols concentration was determined using the calibration curve plotted with gallic acid as standard.

The calibration curve was linear in the range of 2.000 – 16.000 mg/L (R^2 =0.9957). Total phenols content of spices was expressed as mg of gallic acid equivalents per 100 gram of dry weight (mg GAE/100g d.w.). All samples were performed in triplicate and the mean value was reported.

In order to determine the optimal extraction time for the highest total phenols concentration in the spices extractive solutions, the evolution of total phenols was monitored for 5 months period.

Antioxidant capacity (ACL)

The antioxidant capacity was measured using the reagents provided in the ACL kit as per the manufacture's protocol. The ACL assay was performed using Photochem® instrument (Analytic Jena, Germany) against the superoxide anion radicals from luminol, a photosensiter, upon the UV light [24]. In principle the antioxidants from the sample partially eliminates the free radicals and the residual radicals and react with luminol to produce luminescence. The measuring signal (volts) produced by luminescence was traced for specified time duration (120s). The calibration curve was plotted using Trolox as standard (Hoffman – La Roche trade name – 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, which is vitamin E derivative, used for calibration curve), and was quantified as equivalent unit of standard substance. ACL was expressed as nmols Trolox/g dry weight. The obtained extractive solutions were macerated one month before analyze. 5 mL of diluted extractive solution were used for ACL determinations. Three individual measurements were performed and the mean value was reported.

Results and discussions

Superimposed spectra of spice extractive solutions after 14 days to five months have been plotted to establish some correlations with the obtained values of antioxidant properties.

In figures 1-7 are presented the superimposed spectra of spices extractive solutions and it can be observed a continuous increase of the absorbance in time, indicating the enhancement of intensity due to the extractible active compounds increase.

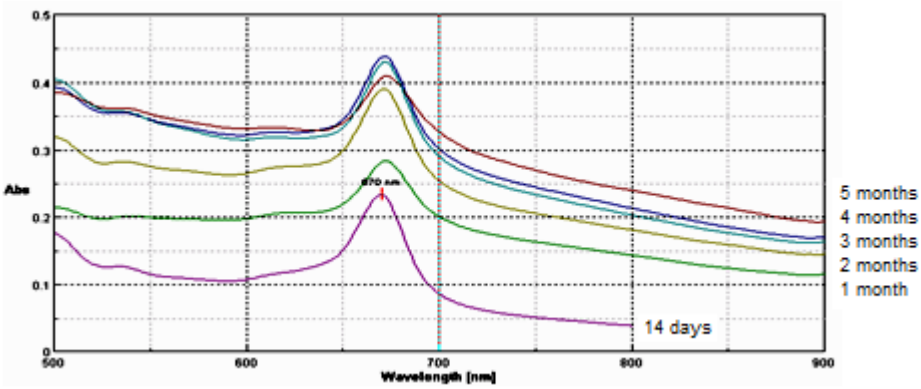

Fig.1. Superimposed spectra of basil (*Ocimum basilicum*) extractive solution in time (14 days to 5 months)

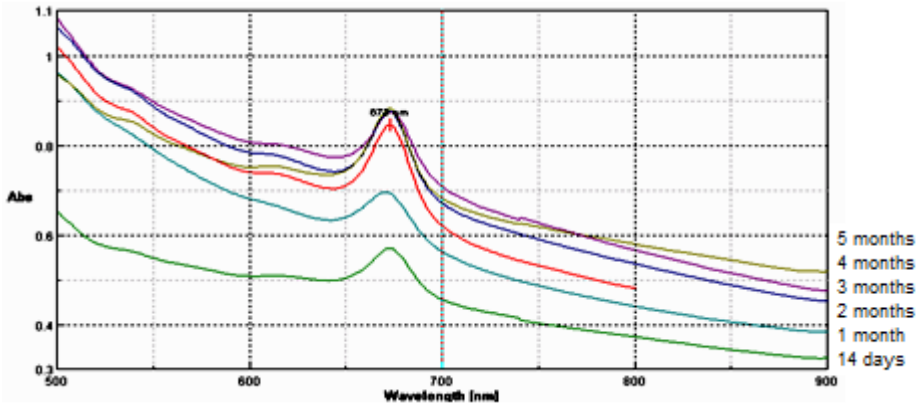

Fig.2. Superimposed spectra of thyme (*Thymus serpyllum*) extractive solution in time (14 days to 5 months)

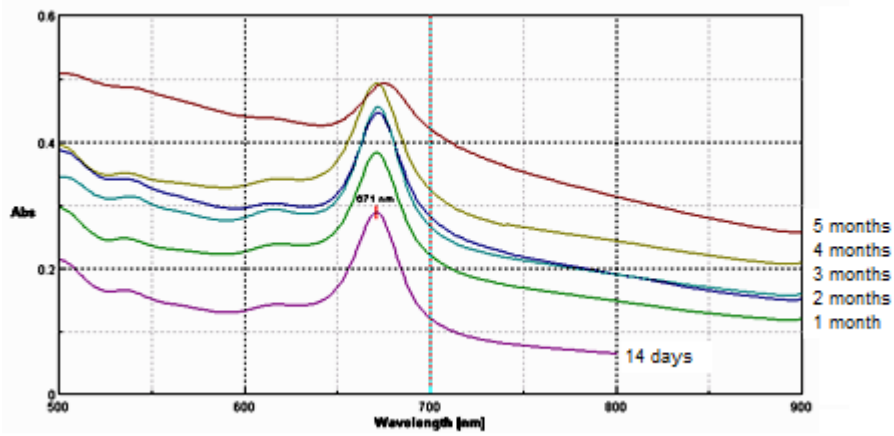

Fig.3. Superimposed spectra of coriander (*Coriandrum sativum*) extractive solution in time (14 days to 5 months)

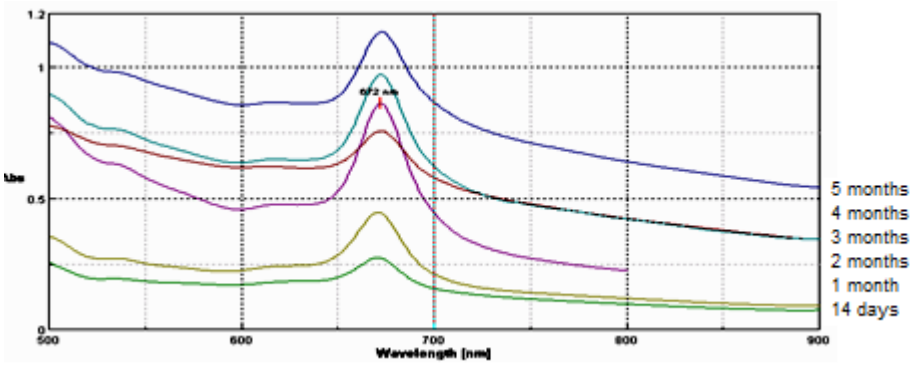

Fig.4. Superimposed spectra of mint (*Mentha piperita*) extractive solution in time (14 days to 5 months)

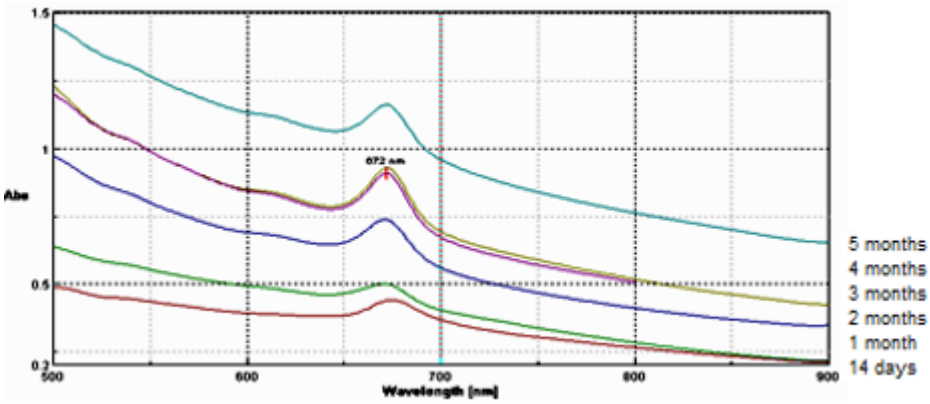

Fig.5. Superimposed spectra of rosemary (*Rosmarinus officinalis*) extractive solution in time (14 days to 5 months)

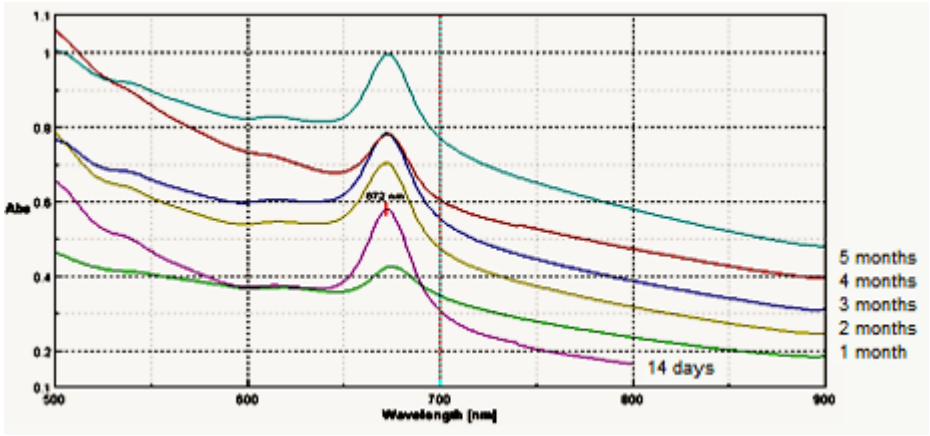

Fig.6. Superimposed spectra of sage (*Sativa officinalis*) extractive solution in time (14 days to 5 months)

Total phenols (TPC)

Results of total phenolic content of extractive solutions form spices expressed as mg GAE/100g dry weight was monitored during a period of 5 months and are presented in table 1.

The first determination done after three days of maceration indicates the highest concentration of phenolic compounds in rosemary (189.72 mg GAE/100g d.w.) followed by thyme (181.30 mg GAE/100g d.w.) and mint (121.72 mg GAE/100g d.w.). The lowest concentration of total phenols was obtained for basil (19.270 mg GAE/100g d.w.).

A high increase of concentration of phenolic compounds from 14 days to one month can be noticed for all investigated spices.

In general the optimal extraction time of the total phenolic content was reached after 3 months of maceration (Table 1), except coriander (one month, 193.12 mg GAE/100 g d.w.), thyme and tarragon (two months, 482.02 GAE/100 g d.w. respectively 171.45 GAE/100 g d.w.).

Total phenols content in alcoholic extracts of studied spices expressed in mg/GAE/100g d.w. (decrease order): rosemary > sage > mint > thyme > coriander > tarragon > basil.

The differences between the highest concentrations of total phenols content in extractive solutions of spices during 5 months can be explained by the chemical composition of the species, plants part used in analyses, growing conditions, harvesting time and location. [25].

The TPC content results (table 1) showed that the average values are significantly different ($p<0.05$) between the analyzed spices. To verify this profile, an ANOVA design (one-way variance analysis) and homogeneity variance test (F_{max} test) were performed. (table 2) All statistical analyses were carried out at the 95% confidence level.

The highest total phenolic content was registered for rosemary (608.37 mg GAE/100 g d.w.), sage (530.66 mg GAE/100 g d.w.) and mint (511.87 mg GAE/100 g d.w.).

For all investigated spices it can be noticed that after reaching the optimum extraction time of total phenols follows a slowly decrease of phenolic content due to degradation reactions of polyphenols over time.

The obtained results are similar with previous published researches concerning the total phenols concentration in spices [5, 8-9, 12, 14].

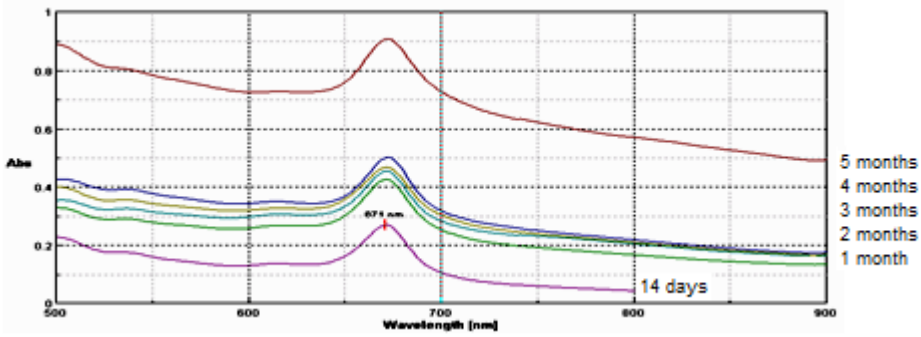

Fig.7. Superimposed spectra of tarragon (*Artemisia dracunculus*) extractive solution in time (14 days to 5 months)

Table 1

EVOLUTION IN TIME OF TOTAL PHENOLIC CONTENT (TPC) IN SPICE ALCOHOLIC EXTRACTIVE SOLUTIONS

Sample/ Time of maceration	TPC, mg GAE/100 g d.w.							
	3 days	7 days	14 days	1 month	2 months	3 months	4 months	5 months
basil	19.270	23.510	48.000	107.25	111.02	121.16	117.84	99.650
thyme	181.30	204.39	274.48	397.32	482.02	446.87	387.84	362.50
coriander	42.160	64.750	108.24	193.12	192.71	179.94	143.18	132.52
mint	121.72	143.52	191.20	353.97	462.71	511.87	490.52	372.54
rosemary	189.72	252.31	309.60	554.77	580.48	608.37	517.16	408.62
sage	74.950	106.39	144.64	368.10	472.71	530.66	490.52	398.23
tarragon	46.310	121.53	145.60	169.40	171.45	167.30	159.84	137.41

Table 2

ANOVA DESIGN FOR THE TPC CONTENT BETWEEN ANALYZED SPICES

Variation sources	Freedom degrees	Sum of squares	Mean squares	Fcrit	P value
Between analyzed spices	6	8.5105	1.4184	9.47	0.00
Error	49	7.3334	1.4966		
Total	55	1.5844			

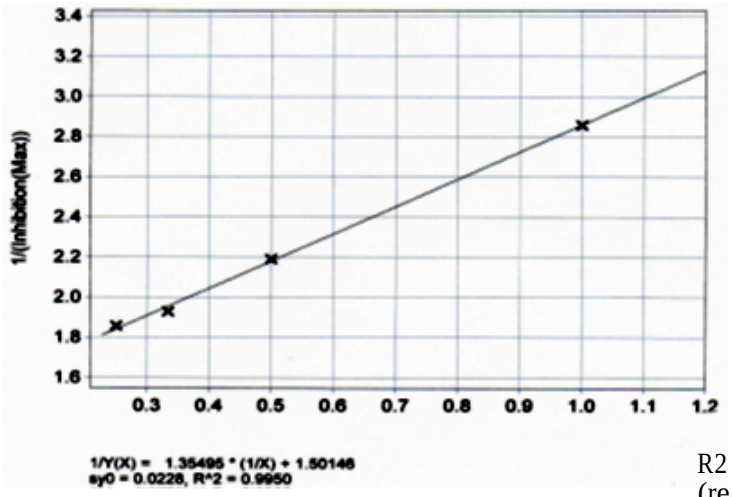

Fig.8. Calibration curve for Trolox in the calculation of Trolox equivalents for ACL measurements (generated by PCLsoft®)

ACL method

By using the ACL method both hydrophilic antioxidants (flavonoids, ascorbic acid) and lypophilic antioxidants (tocopherols, tocotrienols, carotenoids) from the alcoholic extracts of spices can be evaluated [26].

For the calibration curve, standard reagent kit, Analytik Jena Germany Standard was used: R1 (dilution solvent), R2 (buffer reagent), R3 (photosensitive reagent), R4 (reagent sized). The calibration curve was constructed by measuring a series of standard solutions containing 0.5, 1.0, 2.0, 3.0 nmol Trolox (suitable for 5–30mL R4), as in figure 8.

The shapes of antioxidant activities (nmols trolox/g dry weight) of spices extractive solutions measured using PHOTOCHEM apparatus are presented in figure 9.

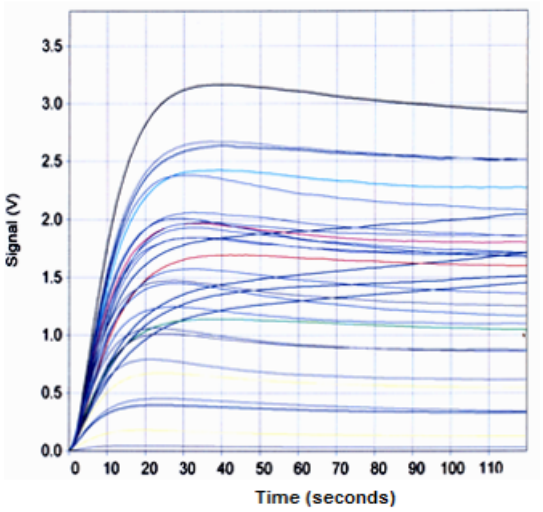

Fig.9. Antioxidant capacity for spices extractive solutions measured using PHOTOCHEM equipment

Table 2
ANOVA DESIGN FOR THE TPC CONTENT BETWEEN ANALYZED SPICES

Variation sources	Freedom degrees	Sum of squares	Mean squares	Fcrit	P value
Between analyzed spices	6	8.5105	1.4184	9.47	0.00
Error	49	7.3334	1.4966		
Total	55	1.5844			

Sample	Sample volum (μL)	Antioxidant capacity Trolox equivalent units (nmols/mL)	Antioxidant capacity Trolox equivalent units (nmols/g d.w.)
Basil	5	27.100	216.80
Thyme	5	24.225	193.80
Coriander	5	2.0710	16.568
Mint	5	7.400	59.200
Rosemary	5	176.25	1410.1
Sage	5	37.025	296.20
Tarragon	5	0.5320	4.2560

Table 3
ANTIOXIDANT CAPACITY OF SPICE EXTRACTIVE SOLUTIONS DETERMINED BY ACL METHOD TROLOX EQUIVALENT UNITS (nmols/g DRY WEIGHT)

The antioxidant capacity, expressed as nmols Trolox/g dry weight ranged between 1410.1 nmols Trolox/g dry weight for rosemary and 4.2560 nmols Trolox/g dry weight for tarragon followed the same pathway as the polyphenolic content did (table 3).

Trolox equivalent units (nmols/g dry weight)

The highest value of the total antioxidant activity was registered in the alcoholic extract of rosemary (1410.1 nmol Trolox/g d.w.) and the lowest in the alcoholic extract of tarragon (4.2560 nmols Trolox/g d.w.).

Based on the obtained results, the total antioxidant capacity expressed in nmols Trolox/g d.w. of the spices alcoholic extracts decrease in order: rosemary > sage > thyme > mint > basil > coriander > tarragon.

This variability is due to the fact that not all antioxidants inhibit free radicals equally. For some antioxidant compounds, their antioxidant activity does not show significant protection against radical HO and $O_2^{\cdot}$ which are responsible for cell damage. Also, the antioxidant activity is influenced by the number and position of substituted OH groups and methoxy glycosidic groups present in flavonoids.

The differences between TPC and ACL obtained values for the studied spices could be explained by their content in organic compounds, other than phenolic compounds, which are responsible for antioxidant capacity. Therefore, it was concluded that there is a direct relationship between type of polyphenolic compounds and their concentration in plants.

Conclusions

The highest total phenolic content was registered for rosemary (608.37 mg GAE/100 g d.w.), sage (530.66 mg GAE/100 g d.w.) and mint (511.87 mg GAE/100 g d.w.).

The decreasing order of antioxidant activity may be given as: rosemary > sage > thyme > mint > basil > coriander > tarragon of spice extractive solutions.

The differences between the highest concentrations of total phenols content of extractive solutions form spices during 5 months can be explained by the chemical composition of the species, plants part used in analyses, growing conditions, collection dates.

There is a direct relation between total phenolic content (TPC) and antioxidant capacity (ACL) for almost all studied spice extractive solutions. Therefore, TPC can be used in this case as a rapid and common method to appreciate the antioxidant capacity.

The content of total phenolic in all seven spices studied indicates them as natural antioxidants sources with strong antioxidant capacity which can be used as a replacement for synthetic antioxidants.

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