THE DETERMINATION OF FLAVONOIDS, THE TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF BASIL SEASONING

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Received 25 October 2012, accepted 21 November 2012

Abstract: *The study aims to determine quantitative and qualitative of the flavonoids, total polyphenols and the antioxidant capacity of the dried basil used in food. The investigations were made on extracts obtained at cold or hot, in water, solutions of methanol, ethanol and complex solvents (methanol - water - acetic acid, methanol – acetone – water – formic acid). The determination on more types of extracts give us information about the availability of bioactive compounds in different conditions, different by temperature, pH, and lypo- or hydrophilic character. The obtained data demonstrate that the bioactive substances analysed are very well represented in basil, and this can explain the observed remarkable antioxidant capacity and interest in the presence of this plant in creation of some aliments with a high biological potential.*

Keywords: *antioxidants, spices, thin layer chromatography, DPPH*

1. Introduction

Oxidative stress, which is caused by free radicals, is considered the cause of many diseases of today civilization: cardiovascular, diabetes, various cancers and aging processes [1]. One of the ways to prevent pathologies mentioned above is an appropriate intake of antioxidants. Antioxidants are chemical compounds that protect cells from the harmful effects of reactive oxygen species (ROS) [2]. Good sources of antioxidants are fruit and vegetables, whole grains, because of the intake of vitamins, minerals, bioflavonoids, components with high antiradical potential [3]. The spices are also a reservoir of concentrated phenolic compounds with antioxidant proprieties, such as polyphenols. Concentrated in only a few grams of material, they can represent the simplest ways to increase the phenolic content and the antioxidant capacity of the daily diet with possible health benefits [4].

In this context, I did experimental studies on basil to determine the amount of phenolic compounds (polyphenols and flavonoids) and to evaluate the antioxidant activity of the spice, given the relationship between the total phenolic compounds and antioxidant activity, as evidenced in numerous studies for other foods [1,5].

Basil (*Ocimum basilicum*), one of the most popular plants, a "bridge" between medicine, gastronomy and tradition, is an annual plant of the *Labiatae* family, with a taste / flavour of sweet – peppery, spicy – hot.

It has various uses both to obtain culinary products or flavoring and seasoning (salads, sauces, fish dishes in Mediterranean cuisine and Asian cuisine especially) and in medicine (being used as a remedy in many diseases: intestinal colic, intestinal bloating, vomiting, flu, colds, dry acute and chronic bronchitis, headache, stomach ulcer, urinary tract

infections, anorexia, diarrhea, colitis fermentation [6].

2. Materials and methods

For comparison, quantitative and qualitative chemical study was performed on extracts from two samples of basil: a working evidence (sample K) obtained by purchasing it from the market and a blank sample (sample L) obtained from the warehouse of company Plantavorel Piatra Neamt.

Because the way meaning is done decisively influences the result and gives a more complete picture of the analyzed compounds content, there were varied extraction conditions, working both at room temperature and a reflux temperature, with several types of solvent extraction, experienced and recommended in the literature:

– with water, cold (S_{1R}) and hot (S_{1C});
– methanolic solution (50:50 v/v) cold (S_{2R}) and hot (S_{2C});
– with ethanol 96^0, cold (S_{3R}) and hot (S_{3C});
– with moisture of methanol - water - acetic acid (90:9:1, v/v/v), cold (S_4);
– with mixture of methanol – acetone – water – formic acid (40:40:19,9:0,1, v/v/v/v), in cold conditions (S_5).

Thus, there were analyzed eight samples for each type of spice: five extracted in cold conditions and three in hot conditions. These were obtained through the collapse of the vegetative material with the corresponding solvent. For this, 2 g of each aerial part of the dry vegetative part was extracted with 20 ml solvent (the report vegetative product / solvent is 1:10) in hot conditions, at the reflux temperature and in cold conditions, at the room temperature. The extractive solutions were filtrated and analyzed concerning the total content of the flavons (experimented in the rutoside) and total polyphenols expressed in gallic acid) and the antioxidant capacity.

For the qualitative analyze of the active principals from the obtained extracts, using the method of thin layer chromatography (TLC) [7, 8].

The reference solutions were rutoside – chlorogenic acid - caffeic acid (E1) and rosmarinic acid (E2).

The chemical quantitative study aimed the quantification through spectrophotometry of the flavonoids and polyphenols from the analyzed samples, knowing the fact that for the spices those essentially contribute to the antioxidant activity [9]. It was used in visible and ultraviolet spectrophotometry.

2.1. The quantitative determination of the flavonoids is based on their property to form, in the presence of the cation Al^{3+}, intern complexes, coloured in vivid yellow, which measure the absorbance at λ= 430 nm. The content of total flavons was expressed in g rutoside / 100 g dried vegetative product (g/100g dvp).

2.2. The determining of the content of total polyphenols was realized through the spectrophotometric method, using the Folin reactive: polyphenolic substances react with Folin reagent (phosphowolframic acid in sodium carbonate medium) forming a blue-coloured complex colorimetry at λ= 760 nm. The content of total polyphenols was expressed in gallic acid.

2.3. The determination of the antiradicalic capacity was made though the determination of the capacity of neutralisation of the radical 2,2 diphenyl –1picrilhidrazil (DPPH) and its transformation in its reduced form by the analyzed vegetative extracts.

The results were expressed in inhibition percents of the DPPH.

There were used to make these determinations the following: spectrophotometers UV-VIS CARY 50, CECIL 2020, CINTRA 101, V-550 Jasco, applicator: CAMAG LINOMAT IV; HPTLC plates silica gel G60F254 Merck (100 x 100, 200 x 100).

There was used various types of reactive:

– for TLC: developer: formic acid - ethyl methyl ketone - ethyl acetate (4:4:1:1); reagent of identification: diphenylborinate methanolic solution of β-ethylamine (NP) and polyethylene glycol 400 (PEG); reference solutions: rutoside – clorogenic acid – caffeic acid, rosmarinic acid;
– for quantitative determination of flavonoids: ethyl alcohol 50%, v/v, 10% sodium acetate (Chemical Company SA) 2,5% aluminum chloride;
– for determination of total polyphenols: Folin reagent, sodium carbonate solution 20%, gallic acid monohydrate;
– for determining the antiradicalic capacity: 2,2 diphenyl – 1 picrilhidrazil (DPPH) from Fluka, quercetol dihydrate (Roth) absolute methanol, dimethyl sulfoxide (DMSO);
All chemicals used were analytically pure.

3. Results and Discussion

3.1. *Determinations by TLC*

Chromatograms were viewed in four stages: three stages to identify the presence of flavonoids and polyphenols (figure no. 1) and a time to identify the antioxidant capacity (figure no. 2):
– view at 254 nm before spraying with identification reagent; the purpose of this analysis is to identify the presence of antioxidant compounds of polyphenols type;

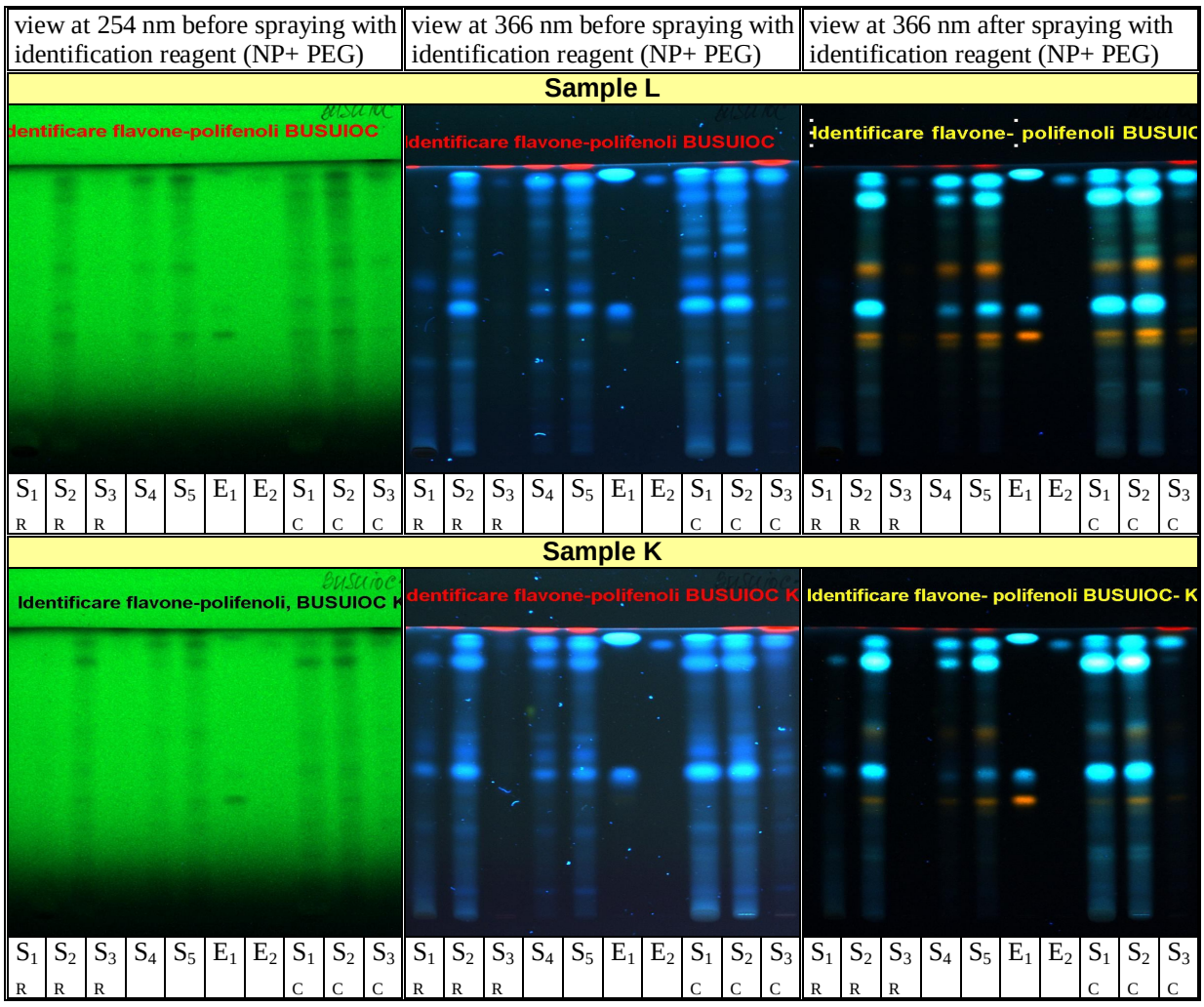

Figure no. 1 – The aspect of the plates and the spots at viewing on thin layer chromatography (TLC) for the flavonoids ad polyphenols in extracts with basil

– view at 366 nm before spraying with identification reagent;
– view at 366 nm after spraying with identification reagent, there are highlighted, depending on position and size of spots, the main types of flavonoids;
– view after spraying with DPPH reagent in order to identify the antioxidant capacity.

Chemical qualitative study conducted by TLC has shown that regardless the extraction solvent and extraction conditions (temperature, extraction technique), the extract obtained solutions were a mixture of phenolic substances (flavonoids and polyphenols), responsible for the antioxidant activity of plant products studied. Thus:

– the occurrence of grey spots – close to samples examined the chromatoplate viewed at 254 nm before spraying with identification reagent, indicating the presence of flavonoid compounds;
– the occurrence of blue spots on the chromatoplate viewed at 366 nm before spraying with identification reagent, indicating the presence of polyphenol carboxylic acids.
– after viewing at 366 nm and spraying with identification reagent are found rutoside, chlorogenic acid and caffeic acid in accordance with standards used. Beside the characteristic spot of the chlorogenic acid, one can find an orange spot that probably belongs to lutein derivatives. Immediately under the characteristic spot of the caffeic acid, we see a blue - fluorescent spot with lower intensity which belongs to all polyphenol carboxylic acids.
- the spots characteristic to flavonoid compounds and polyphenol carboxylic acids suffer a fading action after being sprayed with a solution of DPPH, indicating a consumption of this radical compound by antioxidants in the extracts and their antiradicalic effect.

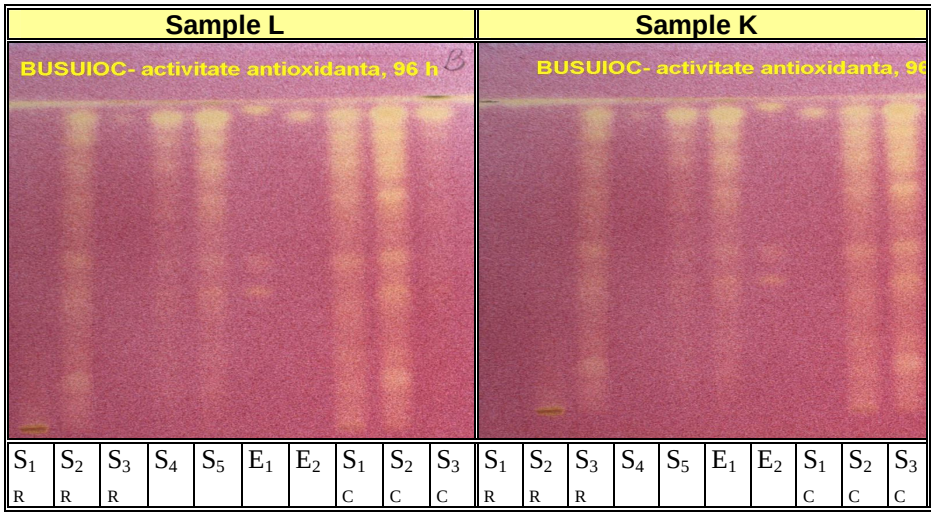

Figure no. 2 – The aspect of the plates and the spots at viewing on thin layer chromatography (CSS) for identify the antioxidant capacity

Comparing the aspect of the correspondent chromatoplates for samples L and K there is established that:

– the content of flavonoid compounds in the sample K is lower than the sample L, although the amount and type of polyphenol carboxylic acids are comparable;
– the antioxidant activity is attributed mostly mainly to polyphenol carboxylic acids;
– the extracts obtained with water, 95%

alcohol respectively, by stirring at room temperature are significantly lower than those obtained using the same solvent extraction, but at reflux temperature.

The optimal extraction of active principles with antioxidant activity is provided with a mixture of methanol / water (50:50), at reflux temperature.

3.2. *Spectrophotometric determinations*

The values obtained at the spectrophotometry dosing of the flavonoids from the samples of spices are various (table no. 1).

Table no. 1
The quantitative determination of the flavonoids from basil extracts

Nr	Solvent (sample)	Flavonoids (g / 100 g dvp)	
		K	**L**
1.	S_{1R}	0,09	0,19
2.	S_{1C}	0,28	0,40
3.	S_{2R}	0,23	0,21
4.	S_{2C}	0,43	0,51
5.	S_{3R}	0,12	0,08
6.	S_{3C}	0,24	0,07
7.	S_4	0,23	0,13
8.	S_5	0,26	0,03

Firstly, one can observe that for sample K the extraction in hot conditions with methanolic solution is the most complete. On the contrary, the extraction in cold conditions with ethanol the value is 4 times less than the maximum (0,43 g / 100 g dvp). The content of flavonoids decreased in extracts in the order: methanol, hot > water, hot > mixture of methanol – acetone – water – formic acid > ethanol, hot > methanol, cold and mixed water – methanol – acetic acid > ethanol, cold > water, cold. At the sample L a different order is observed for lower values. If we compare the values recorded in the determination of flavonoids in tinctures (cold extraction) this will indicate that they are much lower than extracts made at reflux temperature. This is evidence that by increasing the temperature, the extractability of flavonoids is better. Modest results were obtained at the extraction with complex solvents (S_4, S_5).

Comparing the two samples, the blank sample presents superior values for the majority of the types of the extractions. These facts are due to the fact that when purchasing the materials, in the case of the company Plantavorel Piatra Neamt one mainly takes into consideration the bioactive principles and in the case of the materials for obtaining the commercial spices, the accent is based on the sensorial characteristics (mainly on the taste).

Values obtained from spectrophotometric determination of total polyphenols in the two samples have a similar pattern (figure no. 3).

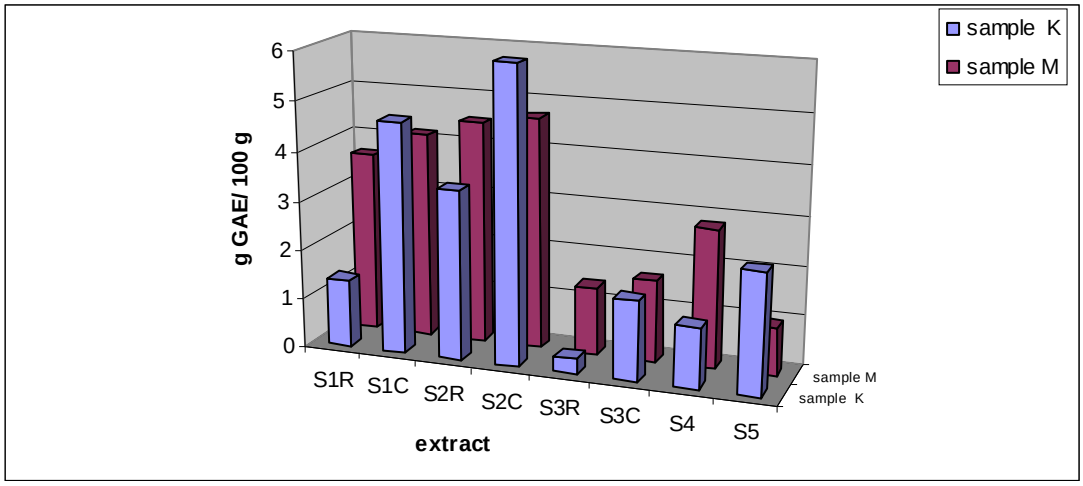

Figure no. 3 –The quantitative determination of polyphenols from basil extracts

The concentration of total polyphenols are in accordance with those in the literature, with one exception: the extract in cold conditions, with ethanol (table no. 2).

Table no. 2
Comparison between the content of polyphenols of the analyzed samples and the data in the literature

Sample	The content of polyphenols of the analyzed samples (g/100 g dvp)		Values from literature (g/100 g dvp)	
	Minimum	Maximum	Minimum	Maximum
K	0,31	5,98	0,740 [10]	6,550 [2]
L	0,98	4,68		

One can observe the wide range of polyphenols content of basil in the date from literature (the difference between the maximum and minimum values is of 5.81 g/100 g dvp). This is because the fact that the analyzes were made on different species of plants, some of them used as spices (due mainly to their intake of volatile oils), some of them in medicinal purposes (because of their bioactive complex), the way of extracting them being different, too.
From the analysis there resulted the fact that the sample obtained in hot conditions with methanolic solution was most effective in „working" with DPPH radicals (table no. 3). The values obtained in determining the antiradicalic capacity are sensible for many of extraction achieved.

Table no. 3
The antioxidant activity of basil extracts, determined through DPPH method

NR	SOLVENT (SAMPLE)	% antiradicalic activity towards the DPPH	
		K	L
1.	S_{1R}	63,55	59,95
2.	S_{1C}	88,70	89,17
3.	S_{2R}	82,40	90,76
4.	S_{2C}	89,48	89,27
5.	S_{3R}	12,01	14,94
6.	S_{3C}	53,23	**91,90**
7.	S_4	4,72	75,1
8.	S_5	75,35	8,14

Is is also noted that there were obtained high values of antiradicalic capacity for extracts wit complex solvents, that is extracts in cold conditions, although the values obtained for polyphenolic compounds have, mostly modest values.

4. Conclusions

From the obtained results we can conclude:
– the extract obtained solutions were a mixture of phenolic substances (flavonoids and polyphenols), as demonstrated by qualitative chemical study performed by TLC and by quantitative measurements;
– flavonoids are extracted more efficiently heat (methanol, and water);
– methanol (and comparable, water), hot, achieved good extraction of polyphenols, both working sample and the blank;
– the antioxidant activity is attributed mostly mainly to polyphenol carboxylic acids (according to qualitative analysis, the most effective "sweeping" radical DPPH hot extracts were performed with methanol and water solution;
– comparing the two samples (K and L), the reference sample presents superior values for the majority of the types of the extractions, so when choosing the materials for obtaining spices one should pay attention to their content in chemical compounds that gives them a high antioxidant potential. In this way it is possible to increase the dietary intake of antioxidants with beneficial effects for health;
- analysed bioactive compounds are very well represented in the basil, but their highlight requires the choice of optimal conditions for extraction.

5. References

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