Estimation of total polyphenols contents in *Ocimum basilicum* L., *Origanum vulgare* L. and *Thymus vulgaris* L. commercial samples

DANIEL MODNICKI*, MACIEJ BALCEREK

Chair of Pharmacognosy
Collegium Medicum
Nicolaus Copernicus University
M. Skłodowskiej-Curie 9
85-094 Bydgoszcz, Poland
*corresponding author: phone/fax: +4852 5853807, e-mail: dmodnicki@wp.pl

Summary

The plants from *Lamiaceae* family are used in medicine, cosmetic and for food as a spice. Phenolic compounds: flavonoids and phenolic acids, the main constituents of this plants have proven multi-directional biological activity. Poyphenols has an antioxidative activity and may act in prevention of many disorders. The estimation of total polyphenols content in commercial samples of *Ocimum basilicum* L., *Origanum vulgare* L. and *Thymus vulgaris* L. herbs, derived from several producers and with different part number, was performed. Total polyphenol contents in plant materials were estimated by means of spectrophotometric method with Folin-Ciocalteu reagent was performed and shown as total polyphenols in dry herb calculated as pyrogallol, gallic acid and caffeic acid. The amount of total polyphenols in *O. vulgare* i *T. vulgaris* herb were similar and higher than in *O. basilicum* herb.

Key words: *Lamiaceae, Ocimum basilicum, Origanum vulgare, Thymus vulgaris, sweet basil, oregano, thyme, polyphenols, polyphenols estimation, Folin-Ciocalteu reagent, spices*

INTRODUCTION

Phenolic compounds are the most widely distributed secondary metabolites, ubiquitous in the plant kingdom. The great majority of active phenolic compounds isolated from higher plants are flavonoids and phenolic acids. Studies on phenolic compounds have shown a wide range of biological activities such as anti-inflammatory, hepatoprotective, antioxidant, antitrombotic, vasodilating and anticarcinogenic. The *Lamiaceae* family plants are also a source of phenolic compounds

of high biological activity. The main classes of phenolic compounds reported to be present in the *Lamiaceae* family are hydroxycinnamic acids (caffeic acid and rosmarinic acid) and flavonoids, mainly in the form of derivatives such as esters and glycosides [1, 2].

Many species of *Lamiaceae* family have been widely used in traditional medicine and as spices in food for centuries. Among aromatic plants belonging to *Lamiaceae* family the most popular ones are sweet basil - *Ocimum basilicum* L., oregano - *Origanum vulgare* L. and thyme - *Thymus vulgaris* L. The herbs of these plants are used in pharmacy, mainly for appetite and digestion stimulation, as diuretic, antispasmodic as well as expectorant and antimicrobial agent [3]. These plants contain essential oils of intensive aroma and may be used both fresh and dried as a spice.

Ocimum basilicum L. is native to India and cultivated in other regions of Asia, Africa and the Mediterranean region. Sweet basil has been in use in the folk medicine for treating dyspepsia, inflammations, cough treatment, diarrhoea, headaches and other pains [3]. The activity is chiefly attributed to a variety of phenolic compounds and composition of essential oil. The main compounds responsible for typical basil aroma are chavicol methyl ether (estragol), linalool, eugenol, 1,8-cineole and methyl cinnamate [3]. The non-volatile compounds were found to be rich in phenolic acids with major part of caffeic and rosmarinic [4].

Thymus vulgaris L. is indigenous to the Mediterranean and neighbouring countries, northern Africa and several parts of Asia. The beneficial properties include antispasmodic and expectorant activities. It has shown antibacterial, antifungal, antivirial, antiprotozoan and antioxidant properties [3]. The chemical composition presents a large number of flavonoids including luteolin, apigenin, naringenin, eriodictyol, cirsilineol, salvigenin, cirsimaritin, thymoine, thymusine, partially present as glycosides, triterpenes: including ursolic and oleanolic acids [3]. The composition of the essential oil can be of many different varieties. Variability is observed among plants growing in different countries. All used varieties of thyme show six components: geraniol, linalool, α-terpineol, carvacrol, thymol and *trans*-thujan-4-ol, terpinen-4-ol [5-7].

Origanum vulgare L. is common throughout Asia, Europe and northern Africa. It has been used as a remedy for respiratory disorders such as coughs, inflammation of bronchial mucous membranes, as expectorant and in dyspepsia, painful menstruation as well as urinary tracts disorders. The active compounds include composition of essential oil: carvacrol, γ-terpinen, *p*-cymene, thymol, and myrcene, α-pinene; flavonoid naringenin and caffeic acid derivatives, in particular rosmarinic acid [3].

The quantity of phenolic compounds present in plants are influenced by genotype, storage method and environmental conditions. For this reason, it is important to determinate the real level of these compounds present in plant of different origin.

The aim of this work was to evaluate the total amount in phenolic compounds of water extracts obtained from several aromatic plants of the *Lamiaceae* family accessible in Polish markets as spices.

MATERIALS AND METHODS

Plant material

A commercial samples of following spices (herb of investigated plants), produced by four manufacturers of Poland were investigated: *Ocimum basilicum* L. (4 samples), *Origanum vulgare* L. (4 samples) and *Thymus vulgaris* L. (3 samples). All samples were available as 10 g pockets and bought at Polish markets in 2007 and 2008. The serial numbers and producers names of the herb samples are available at Chair of Pharmacognosy, Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz.

Methods

Extraction and total polyphenols determination were carried out by means of procedure according to method described in European Pharmacopoeia [8] with modifications – only total polyphenols were determined, measurements of phenolic compounds not adsorbed by hide powder were not performed. The amounts of polyphenols were calculated as pyrogallol (PG), gallic acid (GA) and caffeic acid (CA) equivalents (the latter two of them obtained by calibration curves).

Reagents and equipment

Folin-Ciocalteu reagent (SIGMA-ALDRICH, US) and sodium carbonate (POCH, PL) were used for total phenolic contents estimation. Caffeic acid (CARL ROTH, D) and gallic acid (SIGMA-ALDRICH, US) were used as standards for calibration curves. Pyrogallol (SIGMA-ALDRICH, US) was used as a reference.

WAS 100/X analytical balance (RADWAG, PL), ML 147 water bath (AJL ELECTRONIC, PL) were applied to prepare extracts. U1800 UV spectrophotometer (HITACHI, JP) was used for measuring absorbance. The EXCEL calculating sheet (MICROSOFT, US) was used for calculation.

Extraction

Approximately 0.5 g of herb was weighed accurately on analytical balance and extracted by 150 mL of distilled water for 30 minutes at boiling temperature in water bath. The flask with water extract was cooled down by a stream of running water. A whole content of flask was quantitatively replaced into a calibrated flask and filled up to 250 mL with distilled water. After complete sedimentation of plant material, water extract was percolated through paper filter into another flask. First 50 mL of filtered liquid were rejected. The same procedure was applied for each sample.

Determination of total phenolic compounds

5.0 mL of extract were placed in calibrated flask and filled up to 25 mL by distilled water. 1.0 mL of Folin-Ciocalteu reagent, 10.0 mL of distilled water were added to 2.0 mL of diluted extract (from 25 mL calibrated flask) and filled up to 25 mL by sodium carbonate solution (290 g/L). An absorbance of prepared sample was measured by means of UV spectrophotometer at 760 nm after 30 minutes of incubation in darkness. The same liquid (with pure water instead of plant extract) was used as a blind test. All determinations were performed in triplicate. The content of polyphenols was calculated from the formula:

$$X = \frac{62.5 \times A_1 \times m_2}{A_2 \times m_1},$$

where A_1 – absorbance of pyrogallol solution, A_2 – absorbance of investigated samples, m_1 – mass of investigated sample [g], m_2 – mass of pyrogallol [g].

Solution of reference substance (pyrogallol)

50 mg of pyrogallol was dissolved in distilled water and filled up to 100 mL in calibrated flask. 5.0 mL of obtained solution was diluted in another 100 mL calibrated flask. Absorbance of 2.0 mL of pyrogallol solution (with the adequate reagents) was measured by the same method as described for herb samples.

Calibration curve of gallic acid (GA)

8.6 mg of gallic acid was weighed accurately on analytical balance and dissolved in 10.0 mL calibration flask. 1.0 mL of solution was dissolved in another 10.0 mL calibration flask. For preparing a calibration curve 1, 2, 3 and 4 mL of this solution were placed separately into calibrated flask and filled up to 10 mL. Obtained solutions of gallic acid were: 0.0086, 0.0172, 00258 and 0.0344 mg/mL. 1.0 mL of each solution was mixed with 1.0 mL of Folin-Ciocalteu reagent, 10.0 mL of distilled water and filled up to 25 mL in calibrated flask by sodium carbonate solution. Eventually, the concentrations of standard in the last flask were: 0.000344, 0.000688, 0.001032 and 0.001376 mg/mL. The absorption was read after 30 minutes of incubation at 760 nm. Calibration curve was drawn and the equation was calculated in EXCEL calculating sheet.

The linear correlation between standard concentration and absorbance was expressed as y=f(x), where y – absorbance, x – standard concentration [g/mL].

Equation formula for gallic acid concentration [g/mL] was: y=276793 x and r=0.99808.

Total content of phenolic compounds was calculated by the following formula:

$$X = \frac{5.6450 \times A}{m},$$

where X – total phenolic compounds [%], A – absorbance, m – mass of investigated sample [g].

Calibration curve of caffeic acid (CA)

9.1 mg of caffeic acid was weighed accurately on analytical balance. Preparation of calibration curve was the same as described above. Obtained solutions of caffeic acid were: 0.0091, 0.0182, 0.0273 and 0.0364 mg/mL. Eventually, the concentrations of standard in the last flask were: 0.000364, 0.000728, 0.001092 and 0.001376 mg/mL.

Equation formula for caffeic acid concentration [g/mL] was: y=291535 x and r=0.99989.

Total content of phenolic compounds was calculated by the folowing formula:

$$X = \frac{5.3596 \times A}{m},$$

where X – total phenolic compounds [%], A – absorbance, m – mass of investigated sample [g].

RESULTS AND DISCUSSION

The estimation of the total polyphenols content calculated as equivalents of all three substances used as standards, like pyrogallol, gallic acid and caffeic acid in these culinary herbs available in Poland was not performed so far. There are some references on total amounts of polyphenols described for medicinal herb from *Lamiaceae* family in literature. These data were acquired by means of different methods (eg. ways of extraction, calculating and results presenting).

Total phenolic compounds contents in each investigated sample and respective standard deviations are shown in table 1. The amounts of polyphenols were calculated as equivalents of pyrogallol, gallic acid and caffeic acid. There was a correlation between results obtained by all three calculating methods and total polyphenols contents were higher when were calculated as pyrogallol equivalents than phenolic acids (the differences were approximately from 0.53 to 0.86% for basil, from 0.75 to 0.97% for oregano and from 1.32 to 1.45% for thyme). Small differences (about from 0.04 to 0.17%) between results obtained for gallic acid and caffeic acid were observed and the values calculated as gallic acid equivalents were higher (tab. 1).

Table 1.

Total percentage amount of phenolic compounds

sample number	plant material	total amount of phenolic compounds [% dry weight]		
		in PG eq.	in GA eq.	in CA eq.
1	*Ocimum basilicum*	2.31±0.0384	1.78±0.0183	1.69±0.0174
2		2.75±0.0495	2.21±0.0413	2.10±0.0392
3		2.81±0.0224	2.20±0.0186	2.09±0.0176
4		2.24±0.0804	1.38±0.0797	1.31±0.0757
5	*Origanum vulgare*	3.56±0.0945	2.59±0.0687	2.46±0.0652
6		2.75±0.0813	2.00±0.0579	1.89±0.0550
7		3.16±0.2023	2.22±0.1427	2.18±0.1399
8		4.85±0.1677	3.53±0.1016	3.36±0.0965
9	*Thymus vulgaris*	3.37±0.1769	2.00±0.1052	1.84±0.0926
10		3.56±0.0256	2.11±0.0079	2.00±0.0075
11		3.45±0.1562	2.05±0.0948	1.95±0.0900

Total amount of phenolic compounds in samples of *O. basilicum* herb were ranged from 2.24±0.0804 to 2.81±0.0224 %, from 1.38±0.0797 to 2.21±0.0413 % and from 1.31±0.0757 to 2.10±0.0392% expressed as pyrogallol, gallic acid and caffeic acid equivalents, respectively. Very high contents of total phenols were noticed for *O. vulgare* and *T. vulgaris* herbs. The total polyphenols of *T. vulgaris* herb were ranged from 3.37±0.1769 to 3.56±0.0256% (pyrogallol), from 2.00±0.1052 to 2.11±0.0079% (gallic acid) and from 1.84±0.0926 to 2.00±0.0075% (caffeic acid). Total amounts of phenolic compounds in dry herb of *O. vulgare* ranged from 2.75±0.0813 to 4.85±0.1677%, from 2.00±0.0579 to 3.53±0.1016% and from 1.89±0.0550 to 3.36±0.0965% expressed as pyrogallol, gallic acid and caffeic acid equivalents, respectively.

Total polyphenols contents in examined herbs were earlier estimated by some authors. The results of these studies are difficult to compare with obtained in this work, because of different manners of extraction and calculating methods; some authors gave only the amounts of polyphenols as mass (mg) in weight of dry extract and indicated information about extraction effectiveness (as mg of extract from 1 g of dry plant material). Total polyphenols contents in dry herb were calculated according to data presented by authors in cited literature.

The congruent amounts of polyphenols to presented in this work were found in methanolic extracts of some seasonings [9]. About 121 mg GA/g extract for *O. basilicum*, 150 mg GA/g extract for *O. vulgare* and 210 mg GA/g extract for *T. vulgaris* herbs were determined. The amount of extractable phenols ranged from 100 to 199 mg/g of dry plant material. It means that amounts of total polyphenols in dry plant material were: 1.21–2.41% for *O. basilicum,* 1.50–2.99% for *O. vulgare* and 2.10–4.18% for *T. vulgaris* [9].

The amount of 2.63% total phenolic contents (as GA equivalents) were determinated for 80% methanolic extract from dry herb of *O. basilicum* [10]. A relatively higher value was described in extract obtained after hydrodistillation of plant material. The extraction yield (246 mg/g of dry herb) and total phenols (147 ± 1.60 mg of gallic acid/g extract) declared by authors means that total polyphenols contents was 3.62 % [11].

CONCLUSION

The obtained results show that the total amounts of polyphenols in commercial samples of culinary (and probably remedial, in adequate doses) herb: *O. basilicum*, *O. vulgare* and *T. vulgaris* available in Poland are relatively high, but draw distinctions. The highest amount of polyphenols was determined in *O. vulgare* and *T. vulgaris* herbs. The lowest level of polyphenols was indicated in *O. basilicum* herb.

The known multi-directional activity of this plant-derived substances may suggest beneficial effect of this herbal products for health maintenance. The usage of described herb for seasoning may lead to improving digestion due to its influence on gastric secretion.

ACKNOWLEDGEMENT

This work was financially supported by the *UMK Grant* no.22/2007.

REFERENCES

1. Bruneton J. Pharmacognosy, Phytochemistry. Medicinal Plants. 2nd ed. Londres-New York 1999.
2. Wang H, Provan GJ, Helliwell K. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chem 2004; 87:307-11.
3. PDR for Herbal Medicines, 3rded. Thomson 2004.
4. Zgórka G, Głowniak K. Variation of free phenolic acids in medicinal plants belonging to the *Lamiaceae* family. J Pharm Biomed Anal 2001; 26:79-87.
5. Blum C, Kubeczka KH, Becker K. Supercritical fluid chromatography–mass spectrometry of thyme extracts (*Thymus vulgaris* L.). J Chromatogr A 1997; 773:377-80.
6. Guillén MD, Manzanos MJ. Study of the composition of the different parts of a Spanish *Thymus vulgaris* L. plant. Food Chem 1998; 63:373-83.
7. Fecka I, Turek S. Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoram by chromatographic techniques Food Chem 2008; 108:1039-53.
8. Determination of tannins in herbal drugs in: European Pharmacopoeia 5.0:221.
9. Koşar M, Dorman HJD, Hiltunen R. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chem 2005; 91:525-33.
10. Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem 2007; 102:938-53.
11. Hinneburg I, Damien Dorman H J, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem 2006; 97:122-9.

OCENA CAŁKOWITEJ ZAWARTOŚCI POLIFENOLI W HANDLOWYCH PRÓBKACH *OCIMUM BASILICUM* L., *ORIGANUM VULGARE* L. I *THYMUS VULGARIS* L.

DANIEL MODNICKI*, MACIEJ BALCEREK

Katedra Farmakognozji
Collegium Medicum
Uniwersytet Mikołaja Kopernika
ul. M. Skłodowskiej-Curie 9
85-094 Bydgoszcz
*autor, do którego należy kierować korespondencję: tel./faks: +4852 5853807,
e-mail: dmodnicki@wp.pl

S t r e s z c z e n i e

Rośliny z rodziny *Lamiaceae* wykorzystywane są w lecznictwie, kosmetyce oraz w przemyśle spożywczym, jako przyprawy. Związki fenolowe: flawonoidy i fenolokwasy, będące głównymi składnikami tych roślin, mają udokumentowaną, wielokierunkową aktywność biologiczną. Polifenole wykazują działanie antyoksydacyjne i mogą mieć znaczenie w profilaktyce wielu schorzeń. Przeprowadzono oznaczanie całkowitej zawartości związków polifenolowych w handlowych próbkach przypraw *Ocimum basilicum* L., *Origanum vulgare* L., i *Thymus vulgaris* L. pochodzących od różnych producentów i o różnych numerach serii. Zawartość polifenoli w surowcach oznaczono metodą spektrofotometryczną z zastosowaniem odczynnika Folina-Ciocalteu i przedstawiono w przeliczeniu na pirogalol, kwas galusowy i kwas kawowy. Zawartość związków polifenolowych w zielu *O. vulgare* i *T. vulgaris* była porównywalna i wyższa niż w zielu *O. basilicum*.

Słowa kluczowe: *Lamiaceae, Ocimum basilicum, Origanum vulgare, Thymus vulgaris, polifenole, bazylia, oregano, tymianek, oznaczanie zawartości polifenoli, odczynnik Folina-Ciocalteu, przyprawy*