AGRONOMY SCIENCE
wcześniej – formerly
Annales UMCS sectio E Agricultura
VOL. LXXIII (4) 2018

 http://dx.doi.org/10.24326/asx.2018.4.4

Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin,
S. Leszczyńskiego 58, 20-068 Lublin, Poland
* e-mail: agnieszka.najda@up.lublin.pl

SEBASTIAN BALANT, SEBASTIAN GÓRSKI, AGNIESZKA NAJDA*
MAGDALENA WALASEK

Plant extracts containing phenolic compounds and their antioxidant activity

Ekstrakty roślinne zawierające związki fenolowe i ich aktywność antyoksydacyjna

Summary. The content of polyphenols, flavonoids and antioxidant activity of onion (*Allium cepa* L.), garlic (*Allium sativum* L.), spearmint (*Mentha spicata*) and peppermint (*Mentha × piperita*) extracts were evaluated. All extracts showed a wide range of total polyphenols from 15.10 to 83.90 mg of GAE · 100 g^{-1} of gallic acid equivalent and total flavonoid content from 0.50 to 9.2 mg · 100 g^{-1} of catechin equivalent. Antioxidant activity (AA) was determined by measuring the capture force of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH radical scavenging activity (IC_{50} values) of different extracts ranged from 29.9 to 73.4%. Results of laboratory tests were analyzed statistically using appropriate statistical methods.

Key words: healing potential, species of vegetable plants, herbs

INTRODUCTION

Plants constitute the main source of new pharmaceuticals and health care products. Plant secondary metabolites, such as polyphenols (flavonoids, tannins, stilbenes, coumarins, lignans), can act as free radicals scavengers, metal ion chelators and reducing agents [Goudjil et al. 2015, Derwich et al. 2011, Najda 2017a]. It has been shown that antioxidant activity in plasma increases after eating foods abundant in antioxidants [Cai et al. 2004, Soobrattee et al. 2005]. For this reason, phytochemicals can reduce oxidative stress, maintaining a balance between antioxidants and oxidants. Many medicinal plants have antioxidant properties. Antioxidants extracted from plants in the form of raw extracts or their chemical components are very effective in inhibiting the destructive processes caused by oxidative stress.

In recent years, there has been a growing number of studies focusing on the content determination and identification of phenolic compounds in plant raw materials [Dorman 2003, Soobrattee et al. 2005, Scharbert and Hofmann 2005]. The latest trend of returning to natural sources of medicine in order to maintain health has caused a large development in the acquisition of plant antioxidants. For this reason, this study focuses on polar compounds, such as polyphenolic compounds, that are stable during infusion and storage and are a potential guarantor of a good health [Lee et al. 2003, Najda 2017a, b].

MATERIAL AND METHODS

The research material consisted of whole peppermint herb (*Mentha × piperita* L. var. *officinalis* Sole f. *rubescens* Camus) and spearmint (*Mentha spicata* L.) and consumption part of onion – meaty husk (*Allium cepa* L.) and garlic cloves (*Allium sativum* L.), which were obtained from the agrotechnical experiments of the Department of Vegetable and Medicinal Plants of the University of Life Sciences in Lublin, conducted in 2017. Mint was grown from three-node seedlings planted on experimental plots at the beginning of October 2016. The harvest of plant material (mint herb) was carried out in the mid of July, while onions and garlic were harvested at the beginning of August 2017.

Preparation of plant extracts. Dried samples were ground and sieved. Each sample (20 g) was extracted with 200 mL of 80% ethanol, 80% methanol and 100% distilled water and digested for 1 day at room temperature and then filtered. The solvents were evaporated after concentrating the extracts at 65°C under reduced pressure using a rotary evaporator. Each dry extract was then weighed; the yield of extracts was calculated and stored at 4°C.

The content of polyphenols in air-dry raw materials was determined using the spectrophotometric method [Shahid et al. 2005] with Folin-Ciocalteu reagent at wavelength $\lambda = 765$ nm, expressing the content of polyphenols converted to gallic acid (GAE), flavonoids (spectrophotometric method) according to Christ and Müller [Polish Pharmacopoeia VI 2002], at wavelength $\lambda = 425$ nm, expressing the content calculated as rutin (RE). Furthermore, the antioxidant activity of extracts from the tested raw materials was determined using two tests against DPPH• radical (1,1-diphenyl-2-picrylhydrazyl [Chin et al. 2000].

RESULTS AND DISCUSSION

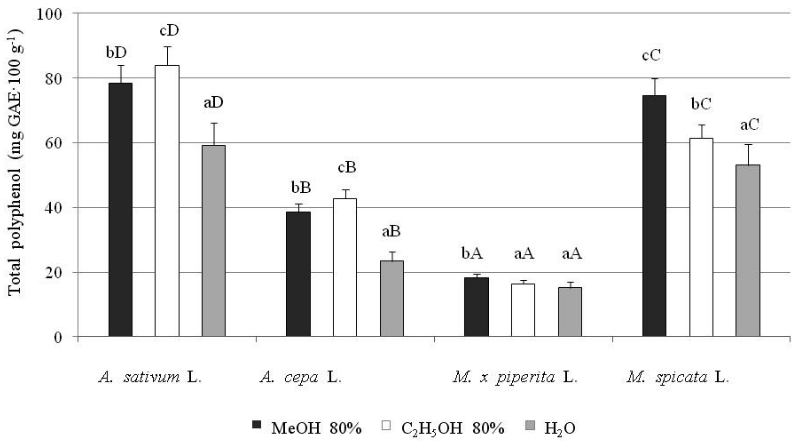
Vegetables and herbs are considered to be an appropriate source of antimicrobial and antioxidant agents. In recent years, many studies have shown that polyphenols present in diet and herbal products inhibit oxidative stress.

Extraction yield

Yields (mg · 100 g^{-1}) of all obtained plant extracts ranged from 5.9 to 12.5 mg · 100 g^{-1}. The maximum yield (12.5 mg · 100 g^{-1}) was obtained with 80% ethanolic garlic extract. Regarding the effectiveness of the solvent, 80% ethanol has been shown to be more effective in recovering antioxidants from herbal plants.

Content of polyphenols

The total polyphenol content in extracts of medicinal plants obtained from four different solvent systems ranged from 83.90 mg GAE · $100g^{-1}$ to 15.10 mg GAE · $100g^{-1}$ p.s.m (Fig. 1). The highest total polyphenol content was determined in garlic extract using 80% ethanol as the solvent (83.90 mg GAE · $100g^{-1}$), while the lowest in the peppermint water extract (15.10 mg GAE · $100g^{-1}$). In the case of mint, higher concentration of polyphenols was obtained using 80% methanol.

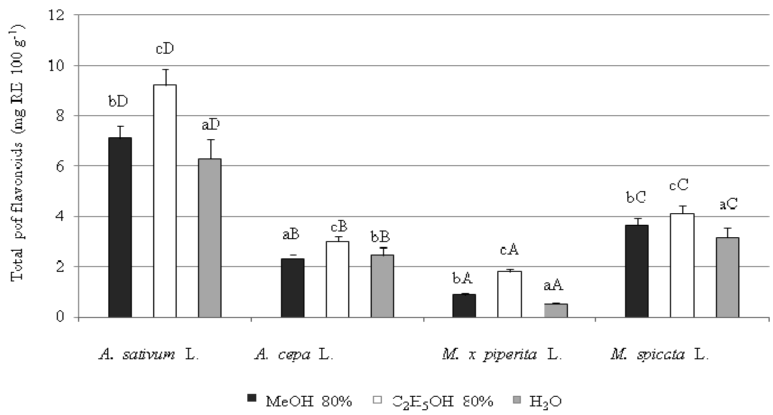


Different letters a, b, c ... indicate statistically significant differences ($p < 0.05$) within the species for different solvents and A, B, C ... indicate statistically significant differences ($p < 0.05$) between species for the same solvent

Fig. 1. Content of total polyphenols in extracts from the raw materials tested

Content of flavonoids

Different levels of flavonoids in extracts from tested raw materials are shown in Fig. 2. The total content of flavonoids in the plant extracts obtained from three solvent systems ranged from 0.50 to 9.20 mg CE · 100 g^{-1}. It was found that 80% ethanolic garlic extract was characterized by the highest content of flavonoid compounds, i.e. 9.20 mg RE · 100 g^{-1}, while the minimum value was 0.50 mg RE · 100 g^{-1} for aqueous peppermint extracts.



Explanations: see Fig. 1

Fig. 2. Content of the sum of flavonoid compounds in extracts from the raw materials tested

Antioxidant activity

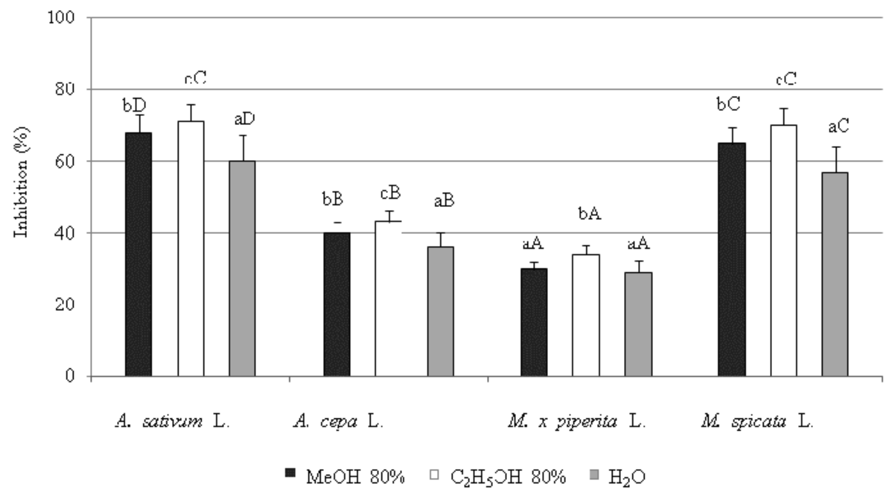
It was found that the antioxidant activity determined in the DPPH test is maximum in 80% ethanolic garlic extract (71.1%), while minimum in the aqueous peppermint extract (28.9%) (Fig. 3). IC_{50} values, that represent the concentration of antioxidants that cause 50% neutralization of DPPH radicals, were calculated from the percentage inhibition versus concentration.

The activity of DPPH radical scavenging (IC_{50} values) ranged from 29.5% to 73.4% (Tab. 1).

Table 1. Neutralization of DPPH radicals (IC_{50}) by extracts from raw materials of various medicinal plants

Species	MeOH 80%	C_2H_5OH 80%	H_2O
M. x piperita L.	67.2 ±0.35^{cB}	39.5 ±0.62^{bA}	42.4 ±0.31^{aA}
M. spicata L.	73.4 ±1.09^{dC}	58.2 ±0.41^{dA}	68.3 ±1.12^{cB}
Allium sativum L.	31.1 ±0.45^{aA}	29.5 ±0.12^{aA}	42.4 ±0.83^{aB}
Allium cepa L.	55.9 ±0.37^{bB}	49.5 ±0.22^{cA}	57.4 ±1.04^{bB}

Values (mean ± SD) of extracts analyzed individually in triplicate
Different letters a, b, c… in the same column and A, B, C… in the same line indicate statistically significant differences ($p < 0.05$)



Explanations: see Fig. 1

Fig. 3. Antioxidant activity measured by DPPH (IC_{50}) test for extracts from the studied species of medicinal plants

It was found that ethanolic garlic extract shows maximum inhibition of peroxidation, which reflects the highest antioxidant activity, while the lowest inhibition was observed in the case of peppermint. With regard to solvents, it has been found that 80% ethanol is more useful for recovering the antioxidant compounds from the raw materials of medicinal plants as compared to other solvent systems.

DISCUSSION

Development of extraction is closely related to the use of this technique not only in the pharmaceutical and chemical, but also in food and biotechnology industries. One of the most important factors in the extraction process is the selection of the right solvent. The choice should be made on the basis of an analysis of many factors that play smaller or larger role in obtaining a good-quality final product [Fink-Finowicki 1955, Brunner 1994].

Results of testing the content of polyphenol fractions in extracts indicate the highest extractive properties of ethanol 80% (in the case of raw onion and garlic) and methanol 80% in extracts from both mint species. Using deionized water, readings were noticeably smaller. This is unusual, because it is generally believed that the polyphenol fraction is a largely hydrophilic fraction – therefore water is used to extract this group [Pieszko and Zaremba 2013]. Ethanol 80% is a readily used solvent in the pharmaceutical industry; many galenic formulations of extracts indicate precisely this concentration as the most suitable for the isolation of biologically active substances [Pluta et al. 2010]. Methanol is more readily used in scientific research, as it is characterized by lower boiling point and similar density and polarity. It should be assumed that both solvents of the same concentration should have identical extractive properties. However, 80% ethanolic extracts are much more abundant in the flavonoid fraction compared to 80% methanol extracts.

Although onions and garlic belong to the genus *Allium*, they do not belong to the same species. In addition, differences between varieties within the species are common, which may justify large differences in the yield between garlic and onions. In the case of mint, the differences in extraction efficiency are directly related to the great variety of this species. The pharmaceutical properties of plant raw materials are closely related to the presence of phenolic compounds (phenolic acids, flavonoids), polysaccharides and proteins [Bianchini and Vainio 2001, Nishimura et al. 2004]. Phenolic acids are an important auxiliary and physiological component of the main active substances.

Nuutila et al. [2003] reported that the total content of polyphenols in extracts of *Allium sativum* L. differed significantly compared to onion extracts and shaped respectively in the range from 845 to 2075 mg $\cdot$ kg^{-1} and from 75 to 115 mg $\cdot$ kg^{-1}. In present study, the total polyphenol content of garlic ethanol extract was the highest (83.90 mg GAE $\cdot$ 100g^{-1}). Lower content of total polyphenols was also found by Benkeblia [2005] when analyzing onion extracts (30 mg $\cdot$ 100 g^{-1} FW), while much higher in garlic extracts (49 mg $\cdot$ 100 g^{-1} FW). Total content of polyphenols in garlic and onion extracts using three solvents decreased in the following order: C_2H_5OH 80% > MeOH 80% > H_2O. Obtained results in relation to garlic extracts are confirmed by El-Hamidi and El-Shami [2015]. The content of these substances in extracts obtained from two species of mint shaped differently. Qadir et al. [2017] reported that the total polyphenol content of peppermint extracts obtained in four different solvent systems was from 9.17 mg GAE · 100 g^{-1} to 14.68 mg GAE · 100 g^{-1}. However, total polyphenol content was the highest in extract with 80% methanol, and the lowest level of these compounds was determined in the aqueous extract. Our results are consistent with these data. Whereas Abdou [2011] found that the total content of polyphenols in garlic extracts was 12.5, 27.6, 23.9 and 25.3 (mg · 100 g^{-1} dry extract), respectively for MeOH, MeOH/H_2O (1 : 1), water at 37°C and water at 100°C.

The total content of flavonoids in plant extracts obtained from four solvent systems ranged from 0.5 to 9.2 mg RE · 100 g^{-1}. The highest total value of flavonoids, regardless of the species, was obtained using 80% ethanol, while the minimum level of these compounds was determined for aqueous extracts. Obtained results were confirmed by Qadir et al. [2017] for garlic, onions and peppermint.

Antioxidant properties of plants are attributed to the active components contained within [Raza et al. 2014, Najda 2017a]. The high antioxidant activity of garlic extract, in particular high level of RAS, has been described in numerous studies [Velioglu et al. 1998, Yin and Cheng 1998, Miller et al. 2000]. However, RAS activity depended on both polyphenolic and sulfur compounds of *Allium sativum* L. Nuutila et al. [2003] found that the lowest antioxidant activity was obtained from garlic extracts, which was confirmed by the results obtained for all solvents used.

CONCLUSIONS

1. Studies have shown that very different extracts of garlic, onion and mint can be obtained by changing the conditions of extraction (solvent), which affect both the efficiency and selectivity of the extraction process.
2. Type of solvent used affects the effectiveness of the antioxidant action of extracts from garlic, onion, peppermint and green mint.
3. 80% ethanol proved to be better flavonoid extractant for all analyzed raw materials.
4. The strongest antioxidant activity was demonstrated by ethanol extracts (towards the DPPH• radical) from garlic and green mint.

REFERENCES

Abdou H.M., 2011. Comparative antioxidant activity study of some edible plants used spices in Egypt. J. Am. Sci. 7, 1118–1122.

Bianchini F., Vainio H., 2001. Allium vegetables and organosulfer compounds: Do they help prevent cancer? Environ. Health Perspect. 109, 893–902.

Benkeblia N., 2005. Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. Braz. Arch. Biol. Techn. 48(5), 753–759.

Brunner G., 1994. Gas Extraction. An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes. Springer, New York.

Cai Y.Z., Luo Q., Sun M., Corke H., 2004. Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer. Life Sci. 74, 2157–2184.

Chin Y.G., Duh P.D., Chuang D.Y., 2000. Antioxidant activity of anthraquinones and anthrone. Food Chem. 70(4), 437–441.

Derwich E., Chabir R., Taouil R.. Senhaji O., 2011. In-vitro antioxidant activity and GC/MS studies on the leaves of Mentha piperita (Lamiaceae) from Morocco. Int. J. Pharm Sci. Drug Res. 3(2), 130–136.

Dorman D.H.J., Koşar M., Kahlos K. et al., 2003. Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties and cultivars. J. Agric. Food Chem. 51, 4563–4569.

El-Hamidi M., El-Shami S.M., 2015. Scavenging activity of different garlic extracts and garlic powder and their antioxidant effect on heated sunflower oil. Am. J. Food Technol. 10(4), 135–146.

Farmakopea Polska VI, 2002. PTF, Warszawa.

Fink-Finowicki C., 1955. Preparatyka galenowa. Zarys farmacji stosowanej [Galenic preparation. Outline of applied pharmacy]. PZWL, Warszawa 7–11, 117–134.

Goudjil M.B., Ladjel S., Bencheikh S.E., Zighmi S., Hamada D., 2015. Chemical compounds profile, antibacterial and antioxidant activities of the essential oil extracted from the Artemisia herba-alba of Southern Algeria. Int. J. Biol. Chem. 9, 70–78.

Lee K.W., Kim Y.J., Lee H.J., Lee C.Y., 2003. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J. Agric. Food Chem. 51, 7292–7295.

Miller H.E., Rigelhof F., Marquart L., Prakash A., Kanter M., 2000. Antioxidant content of whole grain breakfastcereals, fruits and vegetables. J. Am. Coll. Nut. 19, 1–8.

Najda A., 2017a. Zmienność ontogenetyczna mięty (*Mentha species*) czynnikiem warunkującym zawartość składników bioaktywnych w surowcu" [Ontogenetic variability of mint (*Mentha* species) as a factor conditioning the content of bioactive components in the raw material] Rozpr. Nauk. UP w Lublinie 387, Lublin.

Najda A., 2017b. Skład chemiczny i działanie antyoksydacyjne ekstraktów z Mentha x piperita L. [Chemical composition and antioxidant activity of extracts from *Mentha* × *piperita* L.]. Post. Fitoter. 18(4), 252–259.

Nishimura H., Higuchi O., Tateshita K., 2004. Antioxidative activity of sulfur-containing compounds in Allium species for human LDL oxidation in vitro. BioFactors 21, 277–280.

Nuutila A.M., Puuponen-Pimiä T., Aarni M., Oksman-Caldentey K.M., 2003. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem. 81, 485–493.

Pieszko C., Zaremba A., 2013. Zawartość związków fenolowych w ekstraktach z próbek materiału roślinnego [Content of phenolic compounds in extracts from plant material samples]. Bromat. Chem. Toksykol. 46(4), 434–439.

Pluta J., Haznar-Garbacz D., Karolewicz B., Fast M., 2010. Preparaty galenowe [Galenic preparations]. MedPharm Polska, Wrocław 66–77, 103–120.

Qadir M.A., Shahzadi S.K., Bashir A., Munir A., Shahzad S., 2017. Evaluation of phenolic compounds and antioxidant and antimicrobial activities of some common herbs. Int. J. Analyt. Chem., doi.org/10.1155/2017/3475738.

Raza S.A. Rashid A., William J., Razzaq A., 2014. Evaluation of oxidative stability of sunflower oil at frying temperature in presence of butylated hydroxytoluene and methanolic extracts of medicinally important plants of Pakistan. Int. Food Res. J. 21, 331–334.

Scharbert S., Hofmann T., 2005. Molecular definition of black tea taste by means of quantitative studies, taste reconstitution and omission experiments. J. Agric. Food Chem. 53, 5377–5384.

Shahid I., Bhanger M.I., Anwar F., 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem. 93(2), 265– 272.

Soobrattee M., Neergheen V.S., Luximon-Ramma A., Aruoma O.I., Bahorum T., 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mut Res. 579, 200–213.

Velioglu Y.S., Mazza G., Gao L., Oomah B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem., 46, 4113–4117.

Yin M., Cheng W., 1998. Antioxidant activity of several Allium members. J. Agric. Food Chem. 46, 4097–4101.

Research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

Streszczenie. Oceniono zawartość polifenoli, flawonoidów i aktywność antyoksydacyjną ekstraktów z cebuli (*Allium cepa* L.), czosnku (*Allium sativum* L.), mięty zielonej (*Mentha spicata*) i mięty pieprzowej (*Mentha* × *piperita*). Wszystkie ekstrakty wykazały szeroki zakres całkowitej zawartości polifenoli od 15,10 do 83,90 mg GAE 100 g^{-1} równoważnika kwasu galusowego, oraz całkowitej zawartości flawonoidów, średnio 0,50 do 9,2 mg · 100 g^{-1} odpowiednik katechiny. Aktywność przeciwutleniającą (AA) oznaczono przez pomiar siły wychwytywania 2,2-difenylo-1-pikrylohydrazylem (DPPH). Aktywność wychwytywania rodników DPPH (wartości IC_{50}) różnych ekstraktów wahała się od 29,9 do 73,4%. Wyniki badań laboratoryjnych analizowano statystycznie, stosując odpowiednie metody statystyczne.

Słowa kluczowe: potencjał leczniczy, gatunki roślin warzywnych, zioła

Received: 6.09.2018
Accepted: 3.12.2018