medicinska revija
medical review

UDK: 615.322.015.11
COBISS.SR-ID 226163468

Durgo K. et al ■ MD-Medical Data 2016;8(3): 165-167

Originalni članci/
Original articles

EXTRACTION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM MEDICINAL PLANTS-METHODS AND ANTIOXIDANT PROPERTIES

EKSTRAKCIJA BIOLOŠKI AKTIVNIH KOMPONENATA IZ MEDICINSKIH BILJAKA-METODE I ANTIOKSIDANTNA SVOJSTVA

Ksenija Durgo, Marina Lisičar, Ana Belščak-Cvitanović, Ariana Bušić, and Draženka Komes

Faculty of Food Technology and Biotechnology, Zagreb, Croatia

Correspondence to:
Ksenija Durgo,
Faculty of Food Technology and Biotechnology,
University of Zagreb, Pierottijeva 6,
10000 Zagreb, Croatia,
E-mail: kdurgo@pbf.hr
ksenija974@gmail.com

Key words
cell lines, menthe, rosemary, free radicals.

Ključne reči
ćelijske linija, menta, ruzmarin, slobodni radikali

Abstract

Medicinal plants like menthe and rosemary are traditionally used for disease treatment because of the chemical composition and antioxidant capacity. Different methods of extraction will result in different content of bioactive compounds in obtained extracts. Consequently, chemical and biological effect of extracted compounds will differ from one sample to another. Menthe extract has the highest amounts of polyphenols and the highest antioxidant capacity. Menthe extract showed cell type dependend antioxidative/prooxidative nature, while rosmary extract induced free radicals at higher concentrations.

INTRODUCITION

Medical plants are considered to possess a wide variety of curing and protective effects on human health and they have been used in medicinal purposes for centuries. Today it is known that plants can, to a certain level, show protective effects because of the presence of the mixture of biologically active compounds. Generally speaking, medicinal plants are rich of polyphenolic compounds such as flavonoids, phenolic acids, tannins, coumarin or lignin[1]. For a long time it was believed that their activity is lower than synthetic analogues but today scientific community is aware of the fact that there is no definition of low and high concentration and that low concentrations of certain compound, including biologically active compounds can show more potent effect in comparison to higher dosages. Also, one has to be aware that in the natural remedies there is a mixture of biologically active compounds, each of them showing certain biological effect so adequate dosage have positive effect on health without negative side effects[2].

In this work, a brief review of extraction techniques, bioactive compounds and antioxidative potential of two common plants, menthe and rosemary, will be presented.

RESULTS AND DISCUSSION

Preparation of plant extracts and efficiency of extraction often depends upon extraction technique and solvent used. Ratio of extracted polyphenolic compounds will strongly depend on polarity of solvent, time and temperature during extraction process and physic-chemical properties of extracted sample[3].

The influence of extraction method on presence of polyphenolic compounds can be easily seen in two samples of menthe and rosemary extraction; first method includes conventional approach; extraction is conducted by pouring of plant leaves with boiling water, during 15 minutes. Other method presumes extraction during 15 minutes and constant temperature of 80 ºC. After detection of polyphenolic compounds in the prepared extracts it was seen that in the first case the concentration of polyphenols in menthe extract was 60.64 mg EGA g^{-1} and in rosemary extract, 48.84 mg EGA g^{-1}. When other method of extraction was applied content of polyphenolic compounds in menthe extract was significantly higher - 70.04 mg EGA g^{-1}, while different extraction techniques did not play any significant role on polyphenol content in rosemary extract where the concentration of epigallic acid was about 48.04 mg EGA g^{-1}.

If organic solvents are used, the polyphenolic concentration increases significantly. In the case of menthe, extraction with 75 % acetone will result in yield of polyphenolic acids of about 191.8 mg EGK g^{-1} [4]. On the other hand, when other species of menthe were extracted, in water extract of *Mentha pulegium* it was detected of 188 mg EGA g^{-1}, while in the methanolic extract of the same species there was measured only 138 mg EGA g^{-1}. In *Mentha spicata*, it was shown that in methanolic extract there is more polyphenolic content than in acetone extract[5] (76.32 mg EGK g^{-1}vs. 37.84 mg EGK g^{-1}). These results strongly implicate that time and adequate extraction technique must be determined for each species [5]. In the case of rosemary, it can be seen that, beside intrinsic factors in the plant [6,7,8], crucial role on polyphenolic content in rosmery extract will play applied extraction technique [9,10]. Water and methanolic extracts of dried leaves will contain polyphenols in concentration of about 185 mg EGA g^{-1}[11], while this concentration significantly decreased in the methanolic extract prepared from fresh leaves and stem[12]. Solvent play an important role in extraction and quantification of different polyphenolic compounds. For example, water is not selective solvent, since it will dissolve a numerous compounds and hydrolyze a lot of compounds. Hydroalcohols will dissolve only few of compounds, and higher concentrations of alcohols can be used for prevention of extraction of unwanted compounds [13]. Methanol is considered to be a highly effective solvent suitable for polyphenolic compounds extraction [12]. On the other hand, during extraction of bioactive compounds from the dry material, better results will be obtained by solvents which contain water in organic phase [14,15] since water increases diffusion process and improves extraction of polyphenolic compounds out of the plant material.

Antioxidative capacity

Antioxidative capacity of prepared extract depends upon different factors, including type of the matrix, concentration, isomeric form or synergistic effect with other compounds in the extract. According to that, different methods for determination of antioxidative capacity are developed, from chemical to biological.

The most common chemical methods are ABTS•+ and DPPH•. ABTS•+ can be diluted in hydrophilic or lypophilic media so antioxidative nature can be examined through both phases, in dependence of the type of compounds in the extract. Contrary to that, DPPH can be diluted only in organic media, like ethanolwhat represents great limit to interpretation of the role of hydrophilic antioxidants. Antioxidative capacity of plant extracts rich on polyphenolic compounds is connected to their ability to donate hydrogen or electron or to bind free radical [16].

Extracts prepared from menthe showed highest antioxidative capacity and sample prepared by second method of extraction (15 minutes of extraction at 80 ºC) had highest antioxidative potential (2.14mmol Trolox g^{-1}vs. 1.97 Trolox g^{-1}). Expectedly, rosmarinic extract had lower antioxidative capacity in comparison to menthe, but again, extraction on lower temperature resulted in higher antioxidative capacity, what is not suprising since this sample contains more polyphenolic compounds than the sample obtained by pouring of hot water over the leaves (1.52 Trolox g^{-1}vs. 1.34 mmol Troloxg^{-1}). Measurement of antioxidant and prooxidant nature of some extract can be conducted by use of some adequate biological test system. In this work we have exposed 2 human cell lines, larynx and lung cancer cell lines to different concentrations of menthe and rosemary during 1 hour. Obtained results have shown that polyphenols in extract are sensitive compounds and that in dependence of the cell type, concentration or time of exposure they can act as prooxidants inducing production of free radicals. When larynx cells were exposed to menthe extract, antioxidative effect was seen. Contrary, lung cells suffer severe prooxidative damage which was in dose-response manner.

Rosemary extract act as antioxidant at low concentrations and higher concentrations induced free radical formation on both cell lines, once again pointing that polyphenols easily can show prooxidative nature especially at higher concentrations.

According to all of presented data, it can be said that use of plant extracts for preventing or curing certain diseases as a method of traditional or alternative medicine cannot be a substitution for conventional medicinal approach, but represent a source of additional methods which can be used for health improvement and organism regeneration[17].

CONCLUSION

Based on results, it can be said that it is pretty hard to define conditions under which bioactive compounds act as protective ones. Previous thesis: „the more-the better" is brought to question and for majority of cases it is incorrect. Optimal intake of different fruits, vegetables and plant extracts as a source of different bioactive compounds surely will ensure healthy environment to our cells and help in prevention of different diseases caused by unhealthy living environment. Ongoing researches on chemical, physical and biological characteristics of bioactive compounds will give more information about this issue.

Sažetak

Lekovito bilje, poput nane ili ruzmarina tradicionalno se koristi u tretmanu bolesti zbog svog hemijskog sastava kao i antioksidativnog kapaciteta. Različite metode ekstrakcije aktivnih principa ovih biljaka daju i razlike u antioksidativnom potencijalu. Posledično se i hemijski i biološki efekti ekstrahovanih aktivnih principa razlikuju od uzorka do uzorka. Ekstrakt menta ima najveći sadržaj polifenola i stoga najveći antioksidativni kapacitet. Ekstrakt menta takođe pokazuje zavisnu od korišćene kulture ćelija, anti - ili prooksidativnu prirodu, dok ekstrakt ruzmarina indukuje produkciju slobodnih radikala u velikoj meri.

LITERATURE

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

2. Espin J. C., Garcia-Conesa M. T. Tomas-Barberan F. A. Nutraceuticals: facts and fiction. Phytochemistry, 2007; 68: 2986–3008.

3. Bors W., Heller W., Michael C., Saran M. Radical chemistry of flavonoids antioxidants. Advances in experimental medicine and biology, 1990; 264: 165–170.

4. Lv J., Huang H., Yua L., Whent M., Niu Y., Shi H., Wang T. T. Y., Luthria D., Charles D., Yu L. L. Phenolic composition and nutraceutical properties of organic and conventional cinnamon and peppermint. Food chemistry, 2012; 132: 1442–1450.

5. Scherer R., Lemos M. F., Lemos M. F., Martinelli G. C., Martins J. D. L., Silva A. G. Antioxidant and antibacterial activities and composition of Brazilian spearmint (*Menthaspicata* L.). Industrial crops and products, 2013;. 50: 408– 413.

6. Hidalgo P. J., Ubera J. L., Tena M. T., Valcárcel M. Determination of the carnosic acid content in wild and cultivated *Rosmarinus-officinalis*. Journal of agricultultural and food chemistry, 1998;. 46: 2624–2627

7. Del Baño M. J., Lorente J., Castillo J., Benavente-García O., Del Río J. A., Ortuño A. Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinusofficinalis*. Antioxidant activity. Journal of agricultultural and food chemistry, 2003; 51: 4247–4253.

8. Ibañez E., Kubátová A., SeñoránsF. J., Cavero S., Reglero G., Hawthorne S. B. (2003) Subcritical water extraction of antioxidant compounds from rosemary plants. Journal of agricultultural and food chemistry, 2003; 51: 375–382.

9. Albu S., Joyce E., Paniwnyk L., Lorimer J. P., Mason T. J. Potential for the use of ultrasound in the extraction of antioxidants from Rosmarinusofficinalis for the food and pharmaceutical industry. Ultrasonicssonochemistry, 2003; 11: 261–265.

10. Wada M., Kido H., Ohyama K., Kishikawa N., Ohba Y., Kuroda N., Nakashima K. Evaluation of quenching effects of non-water-soluble and water-soluble rosemary extracts against active oxygen species by chemiluminescent assay. Food chemistry, 2003; 87: 261–267.

11. Dorman H. J. D., Peltoketo A., Hiltunen R., Tikkanen M. J. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food chemistry, 2003; 83: 255–262.

12. Shan B., Cai Y. Z., Sun M., Corke H. Antioxidant capacity of 26 spices extracts and characetrization of their phenolic constituents. Journal of agricultural and food chemistry, 2005; 53: 7749-7759.

13. Tomsone L., Kruma Z., Ruta G. Comparison of Different Solvents and Extraction Methods for Isolation of Phenolic Compounds from Horseradish Roots (*Armoraciarusticana*). World academic science, engineering and technology, 2012; 64:903-908.

14. Robards K. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. Journal of chromatology A, 2003;1000: 657-691.

15. Bimakr M., Rahman R. A., Taip F. S., Ganjloo A., Salleh L. M., Selamat J., Hamid A., Zaidul I. S. M. Comparision of different extraction methods for the extraction of major flavonoid compounds from spearmint (*Menthaspicata L.*) leaves. Foodand bioproducts processing, 2011; 89: 67-72.

16. Stoilova I., Krastanov A., Stoyanova A., Denev P., Gargova S. Antioxidant activity of a ginger extract (*Zingiberofficinale*). Food Chemistry, 2007;. 102: 764–770.

17. Jović S., Stambolović V. Traditional complementary and alternative medicine in Serbia: Legalization and integration in health system, MD-Medical data, 2013; 5(4):373-378.

■ The paper was received on 16.09.2016. / Accepted on 21.09.2016.