UDC 664.664 : 633.88

EVALUATION OF THE HEALTH SAFETY OF MEDICINAL PLANTS

Ivan Milovanović[1*], Bojana Beljkaš[1], Jovana Matić[1], Žarko Kevrešan[1], Aleksandra Mišan[1], Marijana Sakač[1], Đorđe Psodorov[1]

[1]Institute for Food Technology, Novi Sad, Serbia

Abstract: *Herbs and spices have been added to foods since ancient times, not only for the purpose of achieving desired aroma, but also for their traditionally known medicinal and food-preserving properties. In recent years, great attention is being given to medicinal plants as an important source of biologically active compounds which have shown antioxidative, antimutagenic and anticarcinogenic effects. Usage of medicinal herbs as components of functional foods is limited by potential presence of harmful pollutants and microorganisms in plant materials.*

In this study, we have evaluated the health safety of certain medicinal plants and spices. The following plants were used as materials: Rhamnus Frangula, Mentha x piperita L., Carum Carvi L., Petroselinum crispum, Origanum Vulgare L., Coriandrum sativum L. i Cynara scolymus, as well as "Vitalplant" and "Gastroherb" medicinal plant preparations. The amounts of toxical and microelements were determined by atomic absorption spectrophotometry. Considering the sometimes large amounts of pesticides used in agriculture, with known harmful effects on human health, presence of traces of chlorinated pesticides was determined in the mentioned plant materials. Evaluation of health safety also included microbiological analysis of the materials and determining the presence of certain strains of bacteria, moulds and yeasts.

Key words: *medicinal plants, toxical elements, microelements, pesticides, microorganisms*

INTRODUCTION

Herbs and spices have been added to foods since ancient times, not only for the purpose of achieving desired aroma, but also for their traditionally known medicinal and food-preserving properties. Lately, attention is payed to the antioxidative potential of medicinal herbs and spices their applications in food production. They can protect food products from lipid peroxidation, which represents one of the most significant chemical changes in foods. Also, antioxidants present in herbs can aid and supplement the existing antioxidative defence mechanisms of the human body (Sies, 1985).

Due to their stability, efficacy and low price, synthetic antioxidants are widely applied in food and cosmetic industry: butylated hydroxyanizole (BHA), butilated hydroxytoluene

*Corresponding author:
e-mail: ivan.milovanovic@fins.uns.ac.rs
Tel: +381 21 485 3837: Fax: +381 21 450725

(BHT), *tert*-butilated hydroquinone (TBHQ) and propylgalate (PG). However, their use in food industry is limited due to doubts of their toxicity, eg. that they can act as promotors of carcinogenesis (Namiki, 1994, Tsuda et al., 1994). Becouse of this, there is a tendency to replace the synthetic antioxidants with natural ones. Herbs, spices and their constituents are considered safe for health, either based on their traditional use without documented harmful effects, or as a result of dedicated toxicology studies (Smid, & Gorris, 1999).

On the other hand, the published research on pesticide residues in crude herbal materials indicate that the presence of chlorinated pesticide residues is quite common. DDT and its derivatives, γ-HCH and other HCH isomers, HCB, and cyclodiene derivatives such as aldrin, dieldrin, heptachlor and its epoxide were reported to occur in these plants (Ali, 1983, Benecke et al., 1986). Many studies have been carried out on spice and medicinal herbs. In Germany, it was reported that the residues of DDT and its derivatives, HCH, dimethoate, and parathion methyl predominated as contaminants (Ali, 1983, Benecke et al., 1986, Schilcher et al., 1987).

Furthermore, they can be contaminated, as are other agricultural products, by heavy metals and microorganisms. Environment conditions in developing countries, pollution from irrigation water, the atmosphere and soil, sterilization methods and inadequate storage conditions all play an important role in contamination of medicinal plants (WHO, 1999). Ingestion of heavy metals through medicines and foods can cause accumulation in organisms, producing serious health hazards such as injury to the kidneys, symptoms of chronic toxicity, renal failure and liver damage (Abou-Arab et al., 1999).

Regarding the fact that *Rhamnus Frangula*, *Mentha x piperita* L., *Carum Carvi* L., *Petroselinum crispum*, *Origanum Vulgare* L., *Coriandrum sativum* L. and *Cynara scolymus* are medicinal herbs that are known to have digestive stimulation action and that are rich in plant phenolics, responsible for their antioxidant activity, these plants could be considered as the functional food components.

The aim of this study was to evaluate the safety of these herbs from the aspect of chlorinated pesticide residues, heavy metals content and microbial count.

MATERIALS AND METHODS

Plant materials:

Medicinal herbs *Rhamnus Frangula*, *Mentha x piperita* L., *Carum Carvi* L., *Petroselinum crispum*, *Origanum Vulgare* L., *Coriandrum sativum* L. and *Cynara scolymus* as well as the mixtures of these medicinal herbs "Vitalplant" (*Rhamnus Frangula* bark (35%), *Mentha x piperita* L. leaves (20%), *Carum Carvi* L. seeds (20%), *Petroselinum crispum* seeds (20%)) and "Gastroherb" (*Origanum Vulgare* L. (55%), *Coriandrum sativum* L. seed (30%), *Cynara scolymus* leaves (15%)), are the products of the Institute of Medicinal Plant Research "Dr Josif Pančić" from Belgrade. The herbal mixtures were in the form of powder, granulation up to 3 mm.

Micro and toxic elements content:

For the sample preparation, dry ashing procedure at 450 °C was applied. The contents of lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), manganese (Mn) and iron (Fe) were determined using the Atomic Absorption Spectrophotometer VARIAN SpectrAA-10, with background correction (D_2-lamp). The content of arsenic (As) was determined using AAS – "hydride generation" technique, on the same apparatus equipped with VGA-76 Vapour Generation Accessory (Pavlović et al., 2001).

Chlorinated pesticide residues:

Samples were prepared for analysis following the procedure recommended by FAO (FAO Food And Nutrition Paper 14/2, 1980). GC-MS was performed with a Thermo Finnigan Trace GC which was connected by a direct interface to a Trace MS Plus Mass Spectrometer. Chlorinated pesticides were separated on a J&W DB-5MS, 60m x 0.25mm I.D. x 0.25μm film thickness capillary column, helium was used as a carrier gas at a constant flow rate of 1 ml/min. Samples were injected automatically by means of a Finnigan AS 2000 autosampler. An injector and the interface temperature were set at 250 °C. The temperature program was: 100 °C (4 min) – 8 °C (1 min) – 200 °C (15 min) – 4 °C (1 min) – 260 °C (2.5 min) – 4 °C (1 min) – 280 °C (6 min). Ion source temperature was set at 200 °C. For calibration supelco pesticides mix, Cat. No.: 4S8913 was used.

Microbiological analysis:

For the microbiological analysis, the dilutions were prepared, and the appropriate culture media were employed for the determination of *Escherichia coli*, *Proteus* sp, *Salmonella* sp., *Sulfitred Clostridia*, *Coagulase positive staphylococci*, moulds, yeasts and total count. The methods used, were according to the Regulation (Pravilnik o metodama obavljanja mikrobioloških analiza i superanaliza životnih namirnica, "Sl. list SFRJ", br. 25/80).

RESULTS AND DISCUSSION

Obtained results for toxic elements are summarized in Table 1. The toxic element concentrations were low in all samples. Lead was detected only in the samples of *Rhamnus Frangula* bark and *Cynara scolymus* leaves while arsenic in the samples of *Rhamnus Frangula* bark, *Cynara scolymus* leaves, *Mentha x piperita* L. leaves, "Gastro-herb" mixture and *Origanum Vulgare* L. Cadmium, considered to be very toxic element, was not detected in any of investigated samples.

Table 1.
Contents of toxic elements in the investigated samples of medicinal plants

Sample	Pb (mg/kg)	Cd (mg/kg)	As (mg/kg)
Rhamnus Frangula bark	1.85±0.30	<0.25	0.09±0.03
Cynara scolymus leaves	1.81±0.27	< 0.25	0.96±0.37
Mentha x piperita L. leaves	< 1.25	< 0.25	0.16±0.02
Coriandrum sativum L. seed	< 1.25	< 0.25	< 0.07
"Vitalplant" mixture	< 1.25	< 0.25	< 0.07
"Gastro-herb" mixture	< 1.25	< 0.25	0.43±0.02
Carum Carvi L. seed	< 1.25	< 0.25	< 0.07
Petroselinum crispum seed	< 1.25	< 0.25	< 0.07
Origanum Vulgare L.	< 1.25	< 0.25	0.21±0.03

Determined amounts of lead and arsenic were lower than the maximum allowed and in accordance with the Regulation (Pravilnik o količinama pesticida, metala i metaloida i drugih otrovnih supstancija, hemioterapeutika, anabolika i drugih supstancija koje se mogu nalaziti u namirnicama, "Sl. list SRJ", br. 5/92, 11/92 i 32/2002).

Some of the obtained results for micro element contents of medicinal plants used in this experiment (Table 2) showed minor differences, when compared with data found in literature (Özcan, 2004). These differences might be due to growth conditions, genetic factors, geographical variations and analytical procedures (Choudhury et al., 2006).

Chlorinated pesticide residues (α-HCH, β-HCH, δ-HCH, Aldrin, Dieldrin, Endosulfan, Endosulfan sulphate, Endrin aldehyde, Endrin ketone, Lindan (γ-HCH), Heptachlor, Heptachlor epoxide, Methoxychlor, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT) were not detected in any of investigated plant mixtures, regarding the detection limit of 0,01 mg/kg.

The results of microbiological analyses are presented in table 3. **Te obtained** results show that "Vitalplant" and "Gastroherb" contained a low number of moulds, below the maximum allowed amount according to the Regulation (Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu, "Sl. list SRJ", br. 26/93). Presence of pathogenic microorganisms was not determined in the investigated samples.

Table 2.
Contents of microelements in the investigated samples of medicinal plants

Sample	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Ni (mg/kg)
Rhamnus Frangula bark	2.66±0.08	5.81±0.05	248±4	137±3	2.93±0.3
Cynara scolymus leaves	7.77±1.05	25.4±0.20	100±1	246±11	3.95±0.2
Mentha x piperita L. leaves	9.95±0.5	18.9±1.10	61±1	197±0.3	1.84±0.2
Coriandrum sativum L. seed	9.25±0.3	33.2±0.10	21.7±0.1	105±1	1.45±0.02
"Vitalplant" mixture	10.1±0.5	20.3±1.44	114±0.1	146±10	2.58±0.1
"Gastroherb" mixture	8.46±0.76	28.3±0.90	65.1±0.7	510±52	5.87±0.2
Carum Carvi L. seed	10.6±0.2	48.4±0.05	22.5±0.2	71.1±8	1.35±0.03
Petroselinum crispum seed	14.3±0.5	36.6±3.0	43.6±0.4	98.9±6	3.36±0.01
Origanum Vulgare L.	9.36±0.60	27.9±1.51	86.1±1.08	257±19	8.41±0.10

Table 3.
Microbiological results for "Vitalplant" and "Gastroherb" mixtures

	"Vitalplant" mixture	"Gastroherb" mixture
Escherichia coli in 0.001 g	nd	nd
Proteus sp. in 0.001 g	nd	nd
Coagulase positive staphylococci in 0.01 g	nd	nd
Sulfitred Clostridia in 0.01 g	nd	nd
Salmonella in 25 g	nd	nd
Moulds in 1 g	150	100
Yeasts in 1 g	0	0
Total number of MO in 1 g	20000	10000

Regarding all the results, it could be concluded that investigated samples of medicinal herbs *Rhamnus Frangula*, *Mentha x piperita* L., *Carum Carvi* L., *Petroselinum crispum*, *Origanum Vulgare* L., *Coriandrum sativum* L. and *Cynara scolymus* as well as the mixtures of medicinal herbs "Vitalplant" and "Gastroherb" are safe from the aspect of chlorinated pesticides residues, heavy metals content and microbial count.

ACKNOWLEDGEMENTS

Original scientific paper was written as a result of work on a project TR20068 “Prehrambeni proizvodi za grupe potrošača sa specijalnim zahtevima i potrebama”, funded by Ministry of Science and Technological Development, Republic of Serbia.

REFERENCES

1. Abou-Arab, A. A. K., Soliman, K. M., El Tantawy, M. E., Ismail, B. R., & Naguid K. (1999). Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chemistry*, *67*, 357–363.
2. Ali, S. L. (1983). Bestimmung der pestiziden Rückstände und anderer bedenklicher verunreinigungen- wie toxische Metallspuren in Arzneipflanzen. 1. Mitt: Pestizid- Rückstände in Arzneidrogen. *Pharmazie Industrial*, 45, 1154–1156.
3. Benecke, R., Brotka, J., Wijsbeek, J., Frank, J. P., Bruins, A. P. & DeZeeuw, R. A. (1986). Untersuchung pflanzlicher Drogen auf Rückstände von organochlorverbindungen. 2. Mittelung. Identifi-

zierung der Rückstände von DDT und semen Analogen durch vergleich von Gaschromatographi an gepackter und kapillars ule sowie GC/MS-Kopplung. *Pharmazie Industrial*, *41*, 499–501.

4. Choudhury, R. P., Kumar, A., & Garg, A. N. (2006). Analysis of Indian mint (*Mentha spicata*) for essential, trace and toxic elements and its antioxidant behaviour. *Journal of Pharmaceutical and Biomedical Analysis*, *41*, 825-832.

5. FAO Food And Nutrition Paper 14/2 (1980), Manuals of Food Quality Control, FAO, Rome.

6. Namiki, M. (1994). Antimutagen and Anticarcinogen research in Japan in Food Phytochemicals I: Fruits and Vegetables. American Chemical Society, 65-81.

7. Özcan, M. (2004). Mineral contents of some plants used as condiments in Turkey. *Food Chemistry*, *84*, 437–440.

8. Pavlović, A., Kevrešan, Ž., Kelemen-Mašić, Đ., & Mandić A. (2001). Micro and toxic elements in skin, meat and gills of grass carp (*Ctenopharyngodon idella*). In *Proceedings of the 8th Symposium on Analytical and Environmental Problems* (pp. 117-121).

9. Pravilnik o količinama pesticida, metala i metaloida I drugih otrovnih supstancija, hemioterapeutika, anabolika i drugih supstancija koje se mogu nalaziti u namirnicama ("Sl. list SRJ", br. 5/92, 11/92 i 32/2002).

10. Pravilnik o metodama obavljanja mikrobioloških analiza i superanaliza životnih namirnica ("Sl. list SFRJ" br. 25/80).

11. Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu ("Sl. list SRJ", br. 26/93).

12. Schilcher, H., Peters, H., & Wank, H. (1987). Pestizide und Schwermetalle in Arzneipflanzen und Arzneipflanzen-zubereitungen. *Pharmazie Industrial*, *49*, 203–211.

13. Sies, H. (1985). Oxidative stress: Introductory remarks. In H. Sies, *Oxidative stress*, pp. 1-8. London: Academic Press.

14. Smid, E. J., & Gorris, L. G. M. (1999). Natural antimicrobials for food preservation. In M.S. Rahman, *Handbook of Food Preservation*, pp. 285–308. New York: Marcel Dekker.

15. Tsuda, T., Ohshima, K., Kawakishi, S. & Osawa, T. (1994). Antioxidative pigments isolated from the seeds of Phaseolus vulgaris L. *Journal of Agricultural and Food Chemistry*, *42*, 248-251.

16. World Health Organization (WHO) (1999), Quality Assurance of Pharmaceuticals. A Compendium of Guidelines and Related Materials. *Good Manufacturing Practice and Inspection,* 2, WHO, Geneva.