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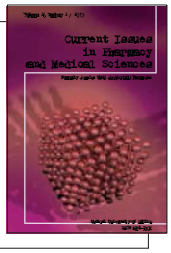

Enzymatic spectrophotometric determination of soluble oxalate content in some multiherbal functional products reducing stress related health disorders

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ABSTRACT

The aim of the study was to determine, with a highly selective and specific enzyme-spectrophotometric procedure, the unknown and not recognized soluble oxalate (SO) content in the aqueous infusions prepared from the randomly chosen series of 15 multiherbal functional products (MFP) commercially available in Poland and recommended for treatment of depression, mood relief and reducing of chronic stress induced by gastric lesions, secondary hypertension or hypercholesterolemia. The mean content of soluble oxalate in the all set of 15 studied MFP products was 1.34 mg/100 mL of the freshly prepared infusion. However, the highest mean content of soluble oxalate (above 2.0 mg/100mL infusion) was determined only in the studied five MFP containing mainly green tea, rooibos, peppermint and ginger rhizome. This highest amount of SO was comparable with previously reported levels of this antinutrient substance determined in case of the commonly drunk green tea infusions. The results of principal components analysis (PCA) revealed a significant relation between unique herbal composition of analyzed dry MFP and the variables characterizing their fresh aqueous infusions as pH, total acidity and level of soluble oxalates.

Keywords: oxalate acid, oxalates, multiherbal functional products, depression

INTRODUCTION

According to the World Health Organization (WHO), the prevalence of depression in populations of many developed countries has been growing during last 20 years, with an estimated 121 million new cases (approximately 5.8% in man and 9.5% in woman) experiencing a depressive episode in any given year. By 2020 year, depression is expected to pose the second greatest health burden of any health disorder in terms of quality of adjusted life years [5, 12]. Also in Poland, since 1989, when the economic transformation began, the rapid and steady growth in the incidence of psychotic and non-psychotic disorders of the nervous system has been observed [13]. Contemporary pharmacotherapy, including selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAO) and tricyclic antidepressants (TCA), could offer a wide range of synthetic antidepressant agents with different degrees of selectivity but still none therapeutic effects have been obtained after the use of these drugs in about 30% of patients with depression [5, 13]. Thus, it is assumed that for a large fraction of Polish population the multiherbal functional products (MFP), as commonly recommended for treatment of depression, mood relief and agents reducing the effects of stress, are easily accessible and rather cheap antidepressants available in pharmacies. Unfortunately, these multiherbal products, consumed in the form of aqueous infusions, contain also a non specified amount of the antinutrient compounds such as oxalic acid and oxalate. Increased every day consumption of ubiquitous oxalate present in most of edible vegetables, fruits and culinary plants can lead to extended risk of oxalosis. This is a metabolic disorder characterized by deposition of oxalate crystals in various organs of human body including cardiovascular arteries and kidney [2, 3, 4, 7]. Diet and nutritional habits are a main environmental risk factor in the idiopathic

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calcium oxalate stone disease, since diet components strongly influence a final urine composition. Patients at risk for calcium oxalate stone formation, especially persons with depressive disorders, are therefore advised to avoid foods and beverages rich in oxalate [14,15]. For patients with kidney stones, *The American Dietetic Association's Nutrition Care Manual* recommends to restrict dietary oxalate intake to less than 40 or 50 mg per day [11]. The role of oxalate ingested by humans from medicinal herbs in the lithogenesis is still unclear. So the aim of this study was to evaluate the levels of soluble oxalate in the fresh aqueous infusion prepared from the set of multi-herbal functional products (MFP) used as the cheap anti-stress agents and distributed in Poland by pharmacy stores, herbal shops and hypermarkets. To our best knowledge in reference literature, there are no data on the oxalate content in this type of stress-reducing multi-herbal functional products.

MATERIALS AND METHODS

The representative samples of randomly selected 15 multi-herbal functional products (MFP), were obtained from the local pharmacy stores and hypermarkets in Bydgoszcz during 2012/13 years. These MFP products were supplied by 6 different Polish manufacturers localized in Dobrzyce, Lublin, Kostrzyń, Lubiszyn, Warszawa and Wrocław. Characteristic data of studied MFP formulations along with their signature and supplier code have been presented in Tab.1. All solvents and reagents were analytical grade and supplied from POCh (Gliwice, Poland). The water infusion was prepared during 5 min using 1.0 g of studied MFP product and 50.0 mL boiling, doubly deionized water. This infusion, after cooling to the room temperature and filtration with membrane filter (pore diameter 40 μm), was analyzed in triplicate for soluble oxalate contents by using selective and specific enzymatic-spectrophotometric assay [11, 14] as obtained from Trinity Biotech (Wicklow, Ireland) and described by Li and Madapally [9]. The method used in our study was two-stage analytical method, based on oxidation of oxalate anion by oxalate oxidase (EC 1.2.3.4) followed by detection of hydrogen peroxide (H_2O_2) which was formed in this reaction. Specifically, this H_2O_2 reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-[dimethylamino] benzoic acid (DMAB) in the presence of peroxidase (EC 1.11.1.7) to form a violet indamine dye which was finally detected spectrophotometrically at 590 nm using spectrophotometer type 1300 (Zeiss, Jena, Germany). A calibration line was constructed in the concentration range of standard dihydrate oxalic acid from 0.20 to 11.00 mg/100mL. Soluble oxalate concentrations were expressed in mg/100mL of aqueous infusion prepared from analyzed MFP formulation. In addition, these results were also expressed in mg/g dry mass (d.m.) of studied MFP (see Table 2). Total acidity (expressed as equivalents of citric acid content mg/100 mL) and pH of each prepared infusion were determined, respectively, by using potentiometric titration with a semiautomatic analyzer DL-22 (Mettler Toledo, Greifensee, Switzerland) and with a desktop microprocessor pH-meter type 213 (Hanna Instruments, Tannieres, France). The six infusions for different batches of each MFP product were prepared individually and analyzed by each of the mentioned procedures. The results of six analytical determinations are given as the mean, standard deviation, minimum and maximum. Statistical differences of between results were calculated by independent *t*-test and coefficient *p* values less than 0.05 were considered as statistically significant. Basic and multivariate statistical (principal component analysis

Table 1. Composition of 15 studied multi-herbal functional products with stress reducing, sedative and mood relief activity

Supplier code	Product code	Plant origin components	Additional components	No.
Dobrzyce	as1	Apple, black tea, blackberry, cola nuts (2%)[a], chicory, guarana seeds (3.4%), hibiscus,mate (5%), rose hip	Aromats, citric acid	11
	as3	Chamomile, birch, fennel, hops, juniper, lemon balm, rose hip, nettle	Aromats	9
	as9	Chamomile (21.4%), blackcurrant, jasmine, lemon balm (3.4%), lemongrass, lemon peel, nutmeg, sage	Aromats	9
Lublin	as2	Hibiscus, bee pollen, blackcurrant, cola nuts (2%), ginseng, mate (8%)	Aromats, ascorbic acid, biotin, caffeine, niacin, pantothenic acid, taurine, vit. B1, vit. B2, vit. B6, vit.E	17
	as5	Black tea, cinnamon, hawthorn (13%), lemon balm (31%), lime, rose hip, verbena	Aromats	8
	as6	Cinnamon bark, guarana seeds (10%), ginger, licoric, rooibos (41%)	Aromats, taurine (2.5%)	7
Kostrzyń	as8	Chamomile (43%), blackcurrant, breakouts, jasmine (2%), lemon balm (7%), lemon peel, nutmeg, sage	Aromats	9
	as11	Coriander, cumin (10%), dandelion, fennel, ginger (5%), mint (55%)	No additives	6
	as12	Apple (7%), asparagus (16.7%), been (3.3%) blackberry, chicory, green tea (10%), hibiscus, lemongrass, lippi leaves, mate, mint	Aromats, citric acid	13
Lubiszyn	as14	Anise, cranberry, hawthorn, ginger (60%), ginseng (3%), green tea, hibiscus, lemon balm, lovage (15%), mate (15%), mint, nettle, rooibos	Aromats	14
	as15	Anise, rose hip, hawthorn, hibiscus, hops (3%), ginseng, lavender, lemon balm (80%), milfoil, mint, primose (5%), sage leaf (10%)	Aromats	13
Warszawa	as7	Apple, chamomile (6.7%), hops (1.7%), lemon balm (15%), rose hip	No additives	5
	as13	Elderberry, cardoon (3.3%), green tea (10.5%), hibiscus (3%), horsetail, mint, peppermint, rose hip	No additives	8
Wrocław	as4	Hawthorn, hops, lavender, lemon balm, lemongrass, licorice	No additives	6
	as10	Apple, chamomile, cumin (60%), mint (7.5%), psyllium seeds fiber (1%)	No additives	5

[a] Content as declared by supplier in weight percent per mass of single sachet. [b] Total number of components in studied multiherbal functional product.

PCA) calculations were carried out using the procedures available in STATISTICA PL v.10 software (StatSoft, Kraków, Polska).

RESULTS AND DISSCUSSION

The 55 different plant origin materials and additives, as declared by the suppliers, were used in various combinations, both in terms of quantity (from 5 to 17 total components in the single MFP product), concentration and quality in composition of the all 15 analyzed here MFP formulations (see Tab. 1). The most commonly used herbal or plant components were lemon balm (in 8 MFP products), mint (7), wild rose hip (6), hibiscus (6), chamomile (5), lemongrass (5), hops (4), hawthorn fruit or flower (4), leaf sage (3), green tea (3), ginger rhizome (3) and ginseng root (3). Limited and selected data on the concentration (from 1 to 80 weight percentage per sachet) of 23 herbal and plant materials were included in the labeling statement of these 15 MFP products. Thus, in the six studied MFP products the single herbal material constituting more than 40% of their contents in single sachet (see Tab. 1), i.e. 80% of lemon balm in the "**as15**" product, 60% of ginger in "**as14**", 60% of cumin in "**as10**", 55% mint in "**as11**", 43% chamomile in "**as8**", 41% rooibos in "**as6**". In the set of studied 15 MFP products up to 10 of them, as declared by the manufacturer, were flavored. Citric acid was used twice ("**as1**" and "**as12**" product) as an acidity regulator and addition of taurine together with ascorbic acid was applied in the single "**as2**" product. In the one MFP with code "**as2**" besides of six plant origin components, also the eight vitamins and caffeine were added (see Tab.1).

The results in Tab. 2 indicated that the studied here MFP were characterized by varying content of soluble oxalate in the range of 0.42 to 2.98 mg/100mL (0.21–1.49 mg/g d.m.). The highest mean content of soluble oxalate 2.86 mg/100mL was determined in MFP product "**as6**" and lowest mean amount 0.49 mg/100mL in product "**as3".** Interestingly, both above mentioned MFP products possessing near identical pH (ca. 5.60) and total acidity (ca. 92.0) of their infusions (see Tab. 2). The pH of infusions obtained from the studied MFP formulations was changed in a range from minimal 3.27 ("**as1**") to maximal 5.83 ("**as11**") and following the shifts in their total acidity from 125.15 mg/100mL to 84.90 mg/100 mL.

All of the results of analytical determinations (pH, total acidity, soluble oxalate) in the studied infusions of 15 MFP (see Tab. 2) were subjected to the multivariate statistics, so-called principal component analysis (PCA), allowing comparison of similarities and dissimilarities of the examined here MFP formulations (see Fig. 1). The first PC1 and second PC2 principal component explained together 97.65% of the total variance of analyzed data from Table 2. Calculated PC1 component expresses the changes in the soluble oxalate content while PC2 component can be related with changes in pH and total acidity of MFP infusions. As result of PCA analysis, the three groups of the studied multi-herbal MFP products could be distinguished:

1) containing 6 products "**as3**","**as7**","**as8**", "**as9**", "**as10**", "**as15**" with a lowest soluble oxalate amount (0.40-1.00 mg/100mL),
2) containing four products "**as1**","**as2**", "**as4**","**as5**" with a medium soluble oxalate amount (1.00-2.00 mg/100mL), and
3) containing five products "**as6**","**as11**","**as12**", "**as13**", "**as14"** with a high soluble oxalate content (2.00-3.00 mg/L). In addition, the clear differentiation of highly acidic ("**as2**","**as1**","**as15**") and low acidic ("**as11**", "**as6**", "**as4**") infusions of studied MFP have been obtained as the result of PCA calculations (see Fig. 1).

It has been shown that the group of studied MFP with the lowest content of SO usually contained chamomile and/or lemon balm, MFP with a medium SO – contained

Table 2. Determined color, pH, total acidity and content of soluble oxalates for studied 15 multi-herbal functional products with stress reducing, sedative and mood relief activity (n =6)

Supplier code	Product code	Color[a]	pH	Total acidity[b] (mg/100mL)	Soluble oxalate (mg/100 mL)		Soluble oxalate (mg/g d.m.)	
					Mean ± SD	Range	Mean ± SD	Range
Dobrzyce	as1	Orange	3.27	125.15	1.43 ± 0.09	1.35 – 1.52	0.72 ± 0.04	0.67 – 0.76
	as3	Amber	5.60	92.00	0.49 ± 0.03	0.46 – 0.51	0.24 ± 0.01	0.23 – 0.26
	as9	Dark yellow	5.01	97.00	0.57 ± 0.04	0.54 – 0.61	0.29 ± 0.02	0.27 – 0.30
Lublin	as2	Amber	3.28	129.50	1.15 ± 0.03	1.12 – 1.18	0.58 ± 0.02	0.56 – 0.59
	as5	Dark rose	5.31	94.65	1.06 ± 0.10	0.96 – 1.15	0.53 ± 0.05	0.48 – 0.58
	as6	Honey like	5.59	90.71	2.86 ± 0.29	2.42 – 2.98	1.43 ± 0.15	1.21 – 1.49
Kostrzyń	as8	Honey like	4.66	103.70	0.67 ± 0.07	0.60 – 0.74	0.34 ± 0.04	0.30 – 0.37
	as11	Light yellow	5.83	84.90	2.03 ± 0.25	1.78 – 2.28	1.02 ± 0.13	0.89 – 1.14
	as12	Dark rose	3.65	124.20	2.42 ± 0.18	2.25 – 2.60	1.21 ± 0.09	1.12 – 1.30
Lubiszyn	as14	Rose	4.49	107.62	2.14 ± 0.35	1.79 – 2.49	1.07 ± 0.18	0.89 – 1.24
	as15	Light yellow	3.36	113.20	0.56 ± 0.01	0.55 – 0.57	0.28 ± 0.04	0.27 – 0.28
Warszawa	as7	Light amber	5.13	97.01	0.45 ± 0.03	0.42 – 0.46	0.23 ± 0.02	0.21 – 0.24
	as13	Dark rose	4.64	103.20	2.31 ± 0.11	2.20 – 2.42	1.16 ± 0.05	1.10 – 1.20
Wrocław	as4	Dark rose	5.50	84.80	1.21 ± 0.08	1.13 – 1.28	0.60 ± 0.04	0.56 – 0.64
	as10	Dark yellow	5.41	90.85	0.63 ± 0.07	0.56 – 0.70	0.31 ± 0.04	0.28 – 0.35
MEAN FOR 15 PRODUCTS:			**4.71**	**102.56**	**1.34**	**0.42 – 2.98**	**0.67**	**0.21 – 1.49**

[a] Color of the freshly prepared water infusion. [b] Calculated as equivalents of citric acid content (mg/100 mL).

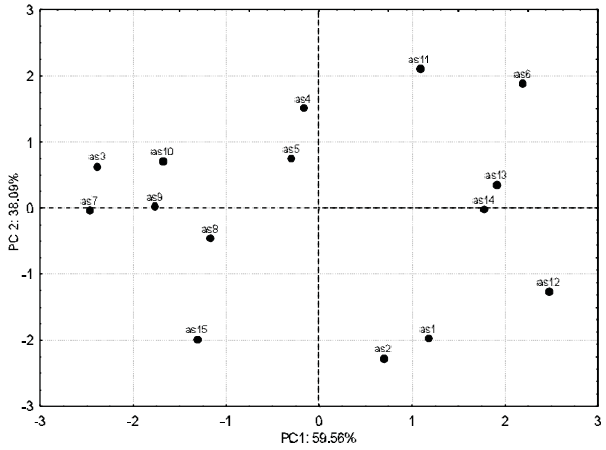

Fig. 1. Loadings plot of the calculated first two principal components, PC1 and PC2, by the determined variables of the 15 studied multi-herbal functional products (pH, total acidity, soluble oxalate content as shown in Tab.1). Symbols on the plane denote code of the each examined multi-herbal product as in Tab. 1 and 2.

rosehip and/or hops, but MFP with high SO – green tea and/or mint (compare Fig.1 and Table 1). However, in the MFP products giving low acidic infusions (pH 5.83) the ginger rhizome is the prevailing plant material. However, hibiscus is the dominating component in case of the MFP formulations giving highly acidic infusions (pH 3.27). These phenomena may also be the consequence of the different proportions of the ingredients in these MFP and can be a result of the region of origin and degree of freshness of plant material used in production of MFP [8]. In Fig. 1, one can observe the superior confirmation of compositional identity of the infusions obtained from the studied here "**as9**" and "**as8**" MFP formulations which contain identical set of plants but differ in their concentration and were made by two different suppliers (see Table 1). Similarly, close proximity of "**as3**" and "**as7**" MFP products on Fig. 1 suggest that their plant composition and content – contrary to the declared recipe - could be similar (see Table 1). It should be pointed out that until now there was published only a few reliable analytical data on the content of soluble oxalate in the aqueous infusions of black and green teas and medicinal herbs which are common phytotherapeutic and pharmacognostic materials. McKay et al. in their study with using the enzymatic method, determined the mean content of soluble oxalate in chamomile infusions at 0.49 mg/100mL, and in the mint infusions – 0.70 mg/100mL [11]. However, Hönow and Hesse, using an enzymatic HPLC procedure, obtained the average content of soluble oxalate in chamomile infusion at 0.30 mg/100mL, mint – 0.60 mg/100mL, nettle – 0.40 mg/100mL, sage – 4.12 mg/100mL, thyme – 2.23 mg/100mL, while in fennel and lemon balm, respectively, 6.00 mg/100mL and 2.65 mg/100mL [7, 8]. Lower results were obtained in studies by Charrier et al., where analyses were made by HPLC with UV detection of oxalate at 210 nm [4]. In their study both chamomile and mint infusions contained 0.16 mg/100mL of soluble oxalate [4]. There is no data in the literature about the soluble oxalate content in other plants as hop, ginger, lemon balm, herb lemongrass and others, used as the components in studied by us MFP products, thus it is difficult to in-depth interpret our results presented in Tab. 2.

CONCLUSION

In conclusion, moderate daily consumption of infusion from MFP, especially prepared from the five MFP stress reducing herbal teas with a detected high content of soluble oxalates, should be encouraged for depressive persons to avoid by them serious unhealthy adverse effects related with an increased consumption of dietary oxalic acid. The results obtained here revealed a statistically significant relation between phytochemical composition of analyzed MFP formulations and the level of soluble oxalates in their infusions. The highest soluble oxalates content were noticed in the MFP products with green tea, peppermint and ginger rhizome. Significantly ($p<0.05$) lower soluble oxalates content was found in the infusions from the MFP products possessing chamomile and lemon balm. The results of study presented here can be used to adjust and optimize the preparation of currently approved recipes of stress reducing multi-herbal functional products due to an unfavorable and high oxalate content and increased acidity.

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