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Original paper

Utilization of microscopic and macroscopic characteristics as part of quality control of herbal teas and tea mixtures and authentication of individual components

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Summary. Herbal teas are preparations that are used for the treatment of mild forms of various diseases, and the quality of these products is of great importance. One of the first checkpoints during quality control of tea involves microscopic and macroscopic control of tea mixtures, which is based on observation and descriptions of the morphology of plant parts in tea mixtures. Quality parameters that rely on microscopic and macroscopic methods (such as adulteration identification, determination of the degree of foreign impurities and authentication) can be easily established, and were shown to be rapid, economically viable and reliable. The main aim of the present study was to point out the importance of utilization of plant cytology, histology and organ morphology in the process of determining the quality level of tea mixtures. In this study, five samples of commercially available tea mixtures were analyzed by means of micro- and macroscopy-based methods, with the aim of content determination, as well as analysis of foreign matter content. Only one of the samples was analyzed for foreign matter content, which was possible due to the properties of the sample (grinding degree). These methods included observation of plant structures with the help of magnifying agents, in order to confirm the presence of plant species in commercial tea mixtures. The obtained results show that four out of five of the analyzed samples contained the herbal drugs listed on the declaration. The degree of foreign impurities in Sample No. 5 (the only one subjected to foreign matter analysis), was 10.4%, which exceeds the allowed limits. The results from this study show that botanical expertise is important during quality control of different raw herbal products, and macro- and micromorphological characteristics represent an important parameter for quality control of these tea mixtures.

Keywords: adulteration, foreign matter, identification characteristics, pharmaceutical botany, pharmacognosy.

INTRODUCTION

Teas are one of the most commonly used herbal remedies, and are found in numerous traditional medicines. Legislation of the European union, the Republic of Serbia and medical documents such as Pharmacopoeias and Herbal Pharmacopoeias categorize teas in different ways (Kovačević 2003; Službeni glasnik RS 4/2012 2012; THIE 2018). Herbal tea mixtures present on the market can be classified into two categories: medical and non-medical. Medical herbal teas are most often used as traditional herbal medicines, and their quality control is performed according to the Pharmacopoeia and valid documents that treat them as such. Non-medical teas are subjected to quality control according to food laws (Kovačević 2003; Vidović et al. 2007; Simmler et al. 2018; Ichim 2019). It is very important that herbal teas meet prescribed quality and safety standards (WHO 1998; Vidović et al. 2007). Teas and other commercial raw herbal products can be obtained without a prescription as OTC ("over the counter") drugs, as well as dietary supplements. Due to the fact that they are easily available and can be of lower quality, their use can pose a threat to consumers who are not aware of this risk (Kumari and Kotecha 2012). It often happens that

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users of herbal tea mixtures do not know the difference between medical and non-medical herbal teas and use both types for therapeutic purposes, according to the principles of traditional medicine (Kovačević 2003; Vidović et al. 2007).

Regardless the category they belong to, herbal teas must meet specific quality and health safety requirements (Vidović et al. 2007). For example, the content must correspond to the declaration (the consumer must not be misled as to the content of the herbal tea mixture), so that the plants and their preparations contained in tea mixture meet the needs of users. Both of these quality parameters can be rapidly evaluated by microscopic and macroscopic methods that rely on knowledge of plant morphology and systematics (Kovačević 2006). Microscopic and macroscopic analyses assist in the determination of the plant composition of herbal tea (Serrano et al. 2010). The analysis process is usually performed by evaluation of morpho-anatomical characters of tea mixture samples and consequent comparison with drug descriptions available in documents provided by the relevant authorities, such as Pharmacopoeias (for official herbal drugs), national and international Directives. In the case of unofficial drugs, which are often used in traditional medicine, characteristics from the botanical literature are used (Kovačević 2006; Serrano et al. 2010). Pharmacognosy, together with pharmaceutical botany, is a fundamental discipline that helps in the first steps of identifying materials of plant origin in tea-type preparations. These steps of quality control are not possible without application of botanical knowledge and skills, especially during analysis of powdered drugs, which represent a special challenge for identification (Kovačević 2006; Evans et al. 2009). Botany and pharmacognosy are closely related, bearing in mind that they originated from scientific studies on medicinal plants (Evans et al. 2009). Literature describing the characteristics used to determine raw plant material, both for official and unofficial drugs, is based directly on the knowledge of biological facts. Plant anatomy and plant systematics provide descriptions of plants and their parts that help to distinguish one species from another. Additionally, along with knowledge of plant ecology and plant genetics, these disciplines provide guidelines and ranges within which given identification characteristics may vary (Evans et al. 2009; Heinrich et al. 2012).

The same methodology can be used for analysis of medical and non-medical herbal teas. However, the content of allowed impurities varies depending on how an herbal tea is declared: no more than 2% is allowed for medicines (for single-component herbal drugs, while there is no clearly defined limit for mixtures), and for non-medical tea the level of total plant parts not listed on the declaration is 1% for teas on the Serbian market, and 2% for teas present on the market of countries of European union (Ph. Jug. IV 1984; Službeni glasnik RS 4/12 2012; Ph. Eur. 8.0 2013; THIE 2018).

The aim of this paper is to emphasize the importance of both microscopic and macroscopic characteristics described in plant taxonomy (including the morphological and microscopical characteristics) for authentication of the contents of herbal teas. As an example, five different tea mixtures are analyzed. Additionally, we aimed to quantify the degree of foreign impurities encountered in one tea mixture that was suitable for this type of analysis. The remaining samples were not suitable for this type of analysis due to their high degree of grinding.

MATERIALS AND METHODS

Samples and sampling process

The samples used in this research were tea mixtures suggested for application in the case of digestive problems (Sample No. 1), upper respiratory tract problems (Sample No. 2), cardiovascular system problems (Sample No. 3), nervousness and anxiety (Sample No. 4) and type 2 diabetes (Sample No. 5) (Table 1, Fig. 1). The tea mixtures were purchased in the Republic of Serbia and the Republic of Poland. The name of each commercially available product as well as the manufacturer has been omitted for protection of the rights of the manufacturer. Samples were stored in the Laboratory of Pharmacognosy at the Faculty of Medicine, University of Novi Sad prior to analysis. All samples were analyzed by microscopic and macroscopic methods in order to identify the separate herbal compounds present in the tea samples.

Macroscopic identification

Macroscopic identification of herbal drugs in the tea mixture was performed according to monographs of the IV Yugoslav Pharmacopoeia (Ph. Jug. IV 1984), the 8th European Pharmacopoeia (Ph. Eur. 8.0 2013) and the standards of the World Health Organization (WHO 1998). Specifically, the analysis included observation of morphological characteristics (shape, size, color, surface properties, texture and other properties) of the grinded herbal samples. The analyses were performed using binocular microscopes, Carl Zeiss AxioLab1 and Stemi 508, both equipped with an AxioCam Erc 5s camera, while the obtained sample images were processed using ZenBlue Software (v3.4)

Microscopic analysis

Prior to microscopic examination, samples were prepared by immersion in a mixture of glycerol and ethanol

Table 1. List of tea samples used for analysis.

Sample No.	Conditions for which tea is intended	Country of origin	Declared composition
1.	Digestive problems	Republic of Serbia	*Chammomilae flos, Matricaria chamomilla* L., Asteraceae *Menthae piperitae folium, Mentha × piperita* L., Lamiaceae *Millefolii herba, Achillea millefolium* s.l., Asteraceae *Coriandri fructus, Coriandrum sativum* L., Apiaceae *Foeniculi fructus, Foeniculum vulgare* Mill., Apiaceae *Anethi fructus, Anethum graveolens* L., Apiaceae
2.	Respiratory tract problems	Republic of Serbia	*Sambuci flos, Sambucus nigra* L., Adoxaceae *Thymi herba, Thymus vulgaris* L., Lamiaceae *Althaeae radix, Althaea officinalis* L., Malvaceae *Chammomilae flos, Matricaria chamomilla* L., Asteraceae *Rabiniae flos, Robinia pseudoacacia* L., Fabaceae *Cynosbati fructus, Rosa canina* L., Rosaceae
3.	Cardiovascular system problems	Republic of Serbia	*Crataegi summitas cum flore, Crataegus monogyna* L., Rosaceae *Tiliae flos, Tilia cordata* Mill., Malvaceae *Cynosbati fructus, Rosa canina* L., Rosaceae *Betulae folium, Betula pendula* L, Betulaceae *Millefolii herba, Achillea millefolium* s.l., Asteraceae *Valerianae radix, Valeriana officinalis*, Valerianaceae *Visci herba, Viscum album* L., Loranthaceae
4.	Mild forms of nervousness and anxiety	Republic of Serbia	*Valerianae radix, Valeriana officinalis*, Valerianaceae *Melissae folium, Mellisa officinalis,* Lamiaceae *Crataegi summitas cum flore, Crataegus monogyna* L., Rosaceae *Menthae piperitae folium, Mentha x piperita* L., Lamiaceae *Millefolii herba, Achillea millefolium* s.l., Asteraceae *Chammomilae flos, Matricaria chamomilla* L., Asteraceae
5.	Intended for use by consumers with diabetes type 2	Republic of Poland	*Myirtilly folium, Vaccinium myrtillus* L., Ericaceae *Radix taraxaci, Taraxacum officinale*, Asteraceae *Phaseoli pericarpium, Phaseolus vulgaris* L., Fabaceae *Callunae herba, Calluna vulgaris* (L.) Hull., Ericaceae *Cynosbati fructus, Rosa canina* L., Rosaceae *Melissae folium, Mellisa officinalis,* Lamiaceae *Leonuri cardiacae herba, Leonurus cardiaca* L. Lamiaceae *Equiseti herba, Equisetum arvense,* Equisetaceae *Rubi folium, Rubus fruticosus* L., Rosaceae *Millefolii herba, Achillea millefolium* s.l., Asteraceae

(1:1) to soften the firmer consistency of herbal drugs. After softening, a section of the drug was prepared, subsequently fixed on a microscope slide, stained with histochemical reagents and observed under microscope (WHO 1998). The histochemical reagent used for visualization in this experiment was Tucakov's general reagent (Tucakov 1984) (Table 2). Tucakov's general reagent is a mixture of chemicals that react with primary and secondary metabolites present in plant material, giving colored products. Each metabolite is present in another compartment of plant tissue or organs. Thus, mixing this reagent and plant material visually arranges plant biomolecules, greatly facilitating visualization of structures that would not be visible under a microscope without the addition of reagents.

Microscopic analysis included examination of the anatomical characteristics of the herbal drugs. This method involves observing the morphology of small plant parts within the tea mixture, with special emphasis on micro-morphological characteristics such as: the structure of plant tissues, the presence or absence of special features such as trichomes, calcium oxalate crystals etc. Observations were performed according to relevant guidelines (Ph. Jug. IV 1984; WHO 1998; Ph. Eur. 8.0 2013). The analyses were performed using Carl Zeiss AxioLab1 and Stemi 508 binocular microscopes, both equipped with an AxioCam Erc 5s camera (WHO 1998; Wani 2007; Kumari and Kotecha 2012; Ph. Eur. 8.0 2013).

Identification of individual components in tea mixtures

After detailed examination of the plant material, the species present in the tea mixture was determined. Identification of plant material was performed using reference litera-

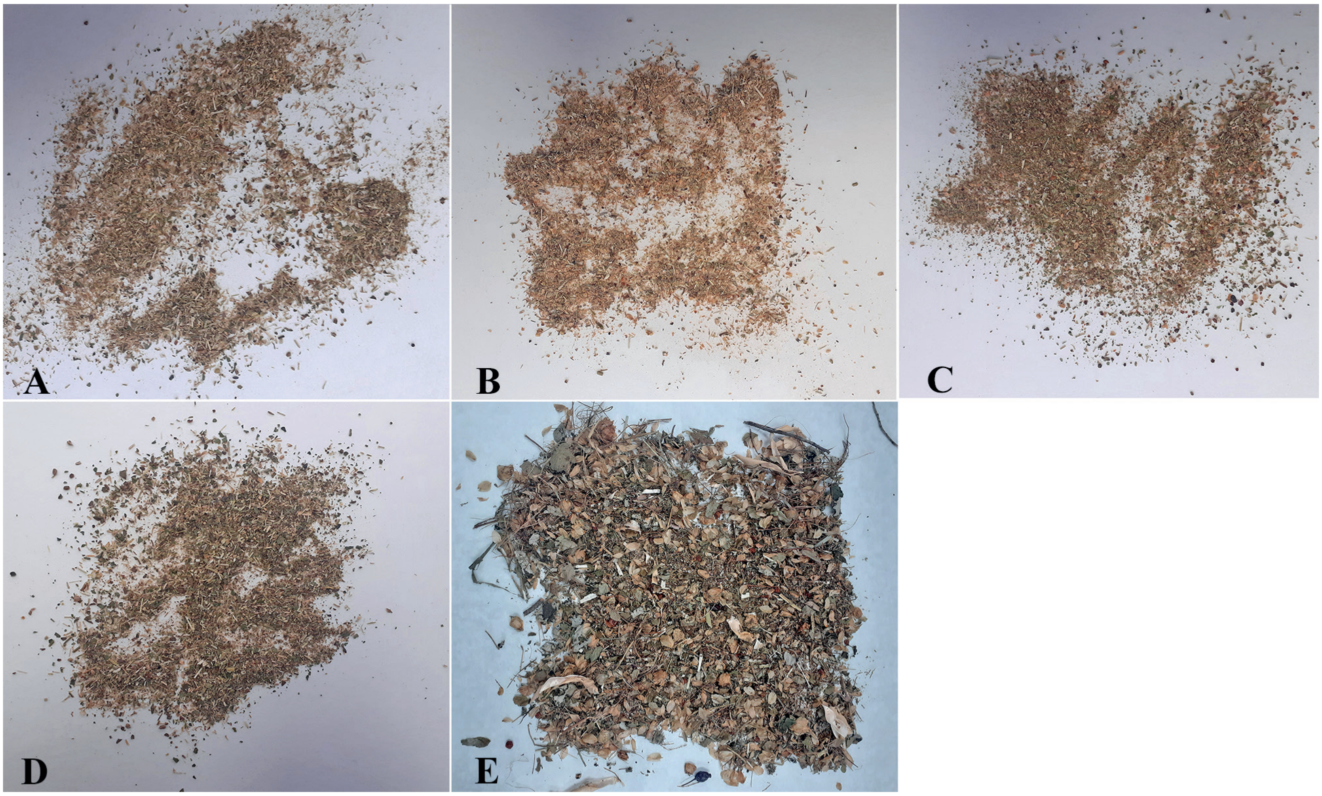

Fig. 1. Photos of tea mixture samples used for analysis. **A**, Sample No. 1 mixture; **B**, Sample No. 2 mixture; **C**, Sample No. 3 mixture; **D**, Sample No. 4 mixture; **E**, Sample No. 5 mixture.

ture, such as Pharmacopoeias and other botanical literature (Josifović 1970–1977; WHO 2009; Upton et al. 2011; Luković and Zorić 2013; Ph. Eur. 8.0 2013; Craig et al. 2018). Species identification involved confirming the identity of a species based on its characteristics (such as tissue micro- and macromorphology). For the purpose of additional confirmation of identity, every feature found in ground parts in a tea mixture were compared with features found in plant material of known and confirmed biological origin (WHO 1998).

Identification and quantification of foreign matter and adulterations

The percentage (%) of foreign matter was determined by physical separation of impurities in 50 g of tea mixture in three replicates (WHO 1998). After microscopic and macroscopic identification of plant fragments within the tea mixture, a list of herbal drugs that are part of the tea mixture was compiled. The list of identified plants was compared to the list available on the product's declaration, and in addition, the correctness of the indications stated in the packaging, which are also related to tea content. The purpose of this comparison was to determine the level of authenticity of the content of tea mixtures. After separation, content of foreign matter was expressed as mass percentage. The content of allowed impurities varies depending on how herbal tea is declared: no more than 2% is allowed for medicines (for single-

Table 2. Composition of "Tucakov's" general reagent used for histochemical analysis of tea mixture components (Tucakov 1984).

Chemical	Formula	Mass/Volume	The purpose of the addition to the mixture
aniline-sulphate	$C_6H_9NO_4S$	0.6 g	visualization of xylem and phloem elements
chloral-hydrate	C_2HCl_3O	0.22 g	fixation of plant cross section
ammonium iron(III) sulfate	$FeNH_4(SO_4)_2 \times 12\ H_2O$	1.2 g	tannins detection
Sudan III reagent	$C_{22}H_{16}N_4O$	0.06 g	visualization of essential oils and fats
iodine	I_2	0.25 g	starch visualization
glycerol	$C_3H_8O_3$	10 ml	rehydration of plant drug
ethanol	C_2H_5OH	15 ml	rehydration of plant drug
distilled water	H_2O	15 ml	solvent

component herbal drugs; there is no clearly defined limit for mixtures); whereas for non-medical teas, the level of total plant parts not listed on the declaration is 1% for teas sold in Serbia, and 2% for teas sold in countries of the European union (Ph. Jug. IV 1984; Službeni glasnik RS, 4/2012 2012; Ph. Eur. 8.0 2013; THIE 2018).

RESULTS

After analysis of samples, identification markers of certain herbal drugs were isolated, and after this step, the given features were photographed and systematically presented in Figures 2-6.

Figure 2. shows the composition of Sample No. 1 mixture. The presence of *Milefolii herba* (yarrow herb), *Chamomillae flos* (chamomile flower), *Foeniculi fructus* (fennel fruit), *Menthae piperithae folium* (peppermint leaf), *Anethi fructus* (dill fruit) and *Coriandri fructus* (coriander fruit) was confirmed in the tea mixture. The presence of yarrow herb was confirmed by observing a lingual flower (Fig. 2A), while the presence of chamomile flower was proven by observation of flower fragments (Fig. 2B) and pollen grains (Fig. 2E) which morphologically show the presence of this drug in Sample No. 1. Fruit fragments of plants from the family Apiaceae (in this case *Foeniculi fructus, Anethi fructus* and *Coriandri fructus*) are shown in Figures 2C, D, G, K and they prove the presence of these drugs in Sample No. 1. The presence of these plants was also confirmed organoleptically (by detecting the characteristic odor that these plants have due to the presence of specific essential oils in them). The analysis of a tea mixture intended for the treatment of digestive problems (Fig. 1) showed the presence of all of the components listed on the product's declaration. According to literature data, dill fruit (*Anethi fructus*), coriander fruit (*Coriandri fructus*), chamomile flower (*Chamomillae flos*), yarrow herb (*Millefolii herba*), fennel fruit (*Foeniculi fructus*) and peppermint leaf (*Menthae piperitae folium*) are used to treat digestive tract problems (WHO 1999a, 2002d, 2007a, 2007b, 2009; Kovačević 2006), so it can be concluded that this tea mixture meets the needs of its users.

The tea mixture intended for treatment of respiratory tract problems, Sample No. 2 (Fig. 3), also contained all components listed on the declaration. Whole flowers of species *Sambucus nigra* (Fig. 3A) were clearly visible in the tea mixture, which confirms the presence of *Sambuci flos* in this mixture, while the stem (Fig. 3C) and petal fragments (Fig. 3B) indicate the presence of thyme (*Thymi herba*) in the tea mixture. The herbal drugs *Sambuci flos* and *Thymi herba* are also found in this tea blend due to the presence of odors of secondary metabolites produced by these plants. The corresponding fragments of parts of the fruit (parts of the flower receptacle and nut type fruit, Figs 3E, F) proved the presence of the drug *Cynosbati fructus*. Root fibers (Fig. 3G) show the presence of marshmallow root (*Althaeae radix*), and flower parts and pollen grains indicate the presence of chamomile flower in Sample No. 2 tea blend. *Robinie flos* (black locust flowers) was identified in this tea mixture intended for respiratory problems, due to the presence of flower fragments (Fig. 3D). All of the listed herbal drugs help with the problems listed on the product declaration, as shown by various literature data (Tucakov 1984; WHO 1999a, 1999b, 2002a, 2002e; Kovačević 2006).

The tea mixture intended for treatment of cardiovascular system problems (Sample No. 3) (Fig. 4) also contained all of the components listed on the declaration. The available data suggest the antihypertensive effects of these herbal drugs (Tucakov 1984; WHO 1999c, 2002b, 2009; Kovačević 2006). The presence of tissue parts of reproductive and vegetative organs (Figs 4O, P, Q) show the presence of *Visci herba* in the tea mixture. Characteristic bracts with specific leaf nerves, parts of corolla and petiole (Figs 4H, I, J) can be observed in parts of the crushed material, which prove that the drug *Tiliae flos* is in Sample No. 3. The presence of this drug was further confirmed by the presence of seeds (Fig. 4K). Fruit fragments (Figs 4A, B) indicate the presence of *Cynosbati fructus*, and fragments of vegetative organs (Fig. 4S, T) and flowers (Fig. 4R) indicated the presence of yarrow herb. Lamina of leaf and leaf petiole fragments (Figs 4C, D) confirm the presence of birch leaves (*Betulae folium*) in the mixture. Fragments of pericarp, stems and seeds (Figs 4E, F, G) indicate the presence of the drug *Crategi fructus* in Sample No. 3 mixture. Valerian root (*Valerianae radix*) was also present in the tea mixture, which is indicated by parts of the root of appropriate morphology (Figs 4L, M, N).

The tea mixture intended for treatment of mild forms of nervousness and anxiety (Sample No. 4, Fig. 5) also contained all of the components listed on the declaration. All of the components present in Sample No. 4 have anxyolitic effects (WHO 1999a, 1999c, 2002b, 2002c, 2002d). In this described tea mixture, the presence of parts of vegetative organs (Figs 5A, B) indicate the presence of *Milefolii herba*. Root fragments (Fig. 5C) indicate the presence of valerian root (*Valerianae radix*), and a non-gladular trichoma associated with tree parts (Fig. 5F) indicates the presence of *Folium cum flore crategi* (Fig. 5D). The herbal drug *Menthae piperithae folium* (peppermint leaf) was obviously present due to the fact that the tea mixture contains microscopically noticeable fragments of leaves with characteristic anatomy associated with non-glandular trichomes merged with the epidermis (Figs 5E, H), as well as the characteristic smell of menthol. Pollen grains with the appropriate architecture of intine and exine confirm the presence of chamomile in the tea mixture (Fig. 5G). Lemon balm leaves (*Mellisae folium*) were con-

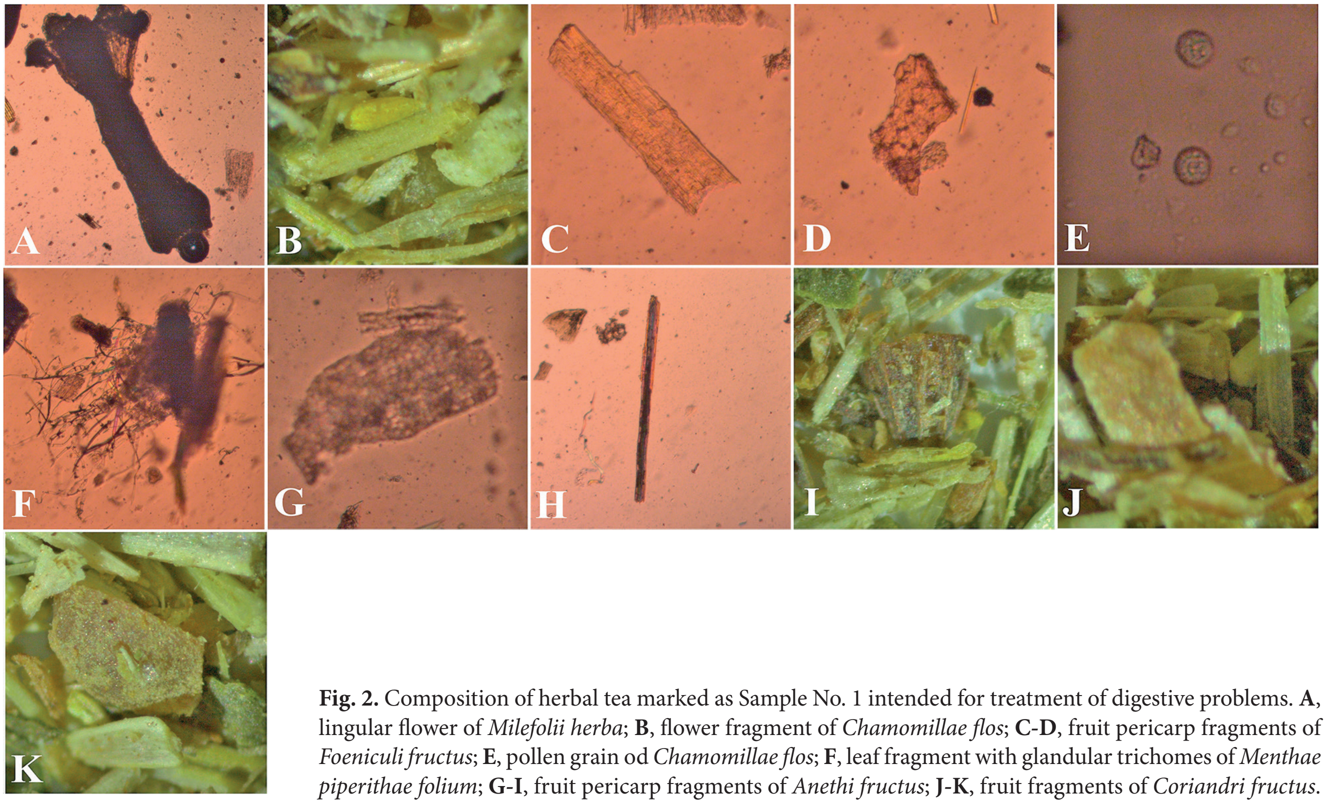

Fig. 2. Composition of herbal tea marked as Sample No. 1 intended for treatment of digestive problems. **A**, lingular flower of *Milefolii herba*; **B**, flower fragment of *Chamomillae flos*; **C-D**, fruit pericarp fragments of *Foeniculi fructus*; **E**, pollen grain od *Chamomillae flos*; **F**, leaf fragment with glandular trichomes of *Menthae piperithae folium*; **G-I**, fruit pericarp fragments of *Anethi fructus*; **J-K**, fruit fragments of *Coriandri fructus*.

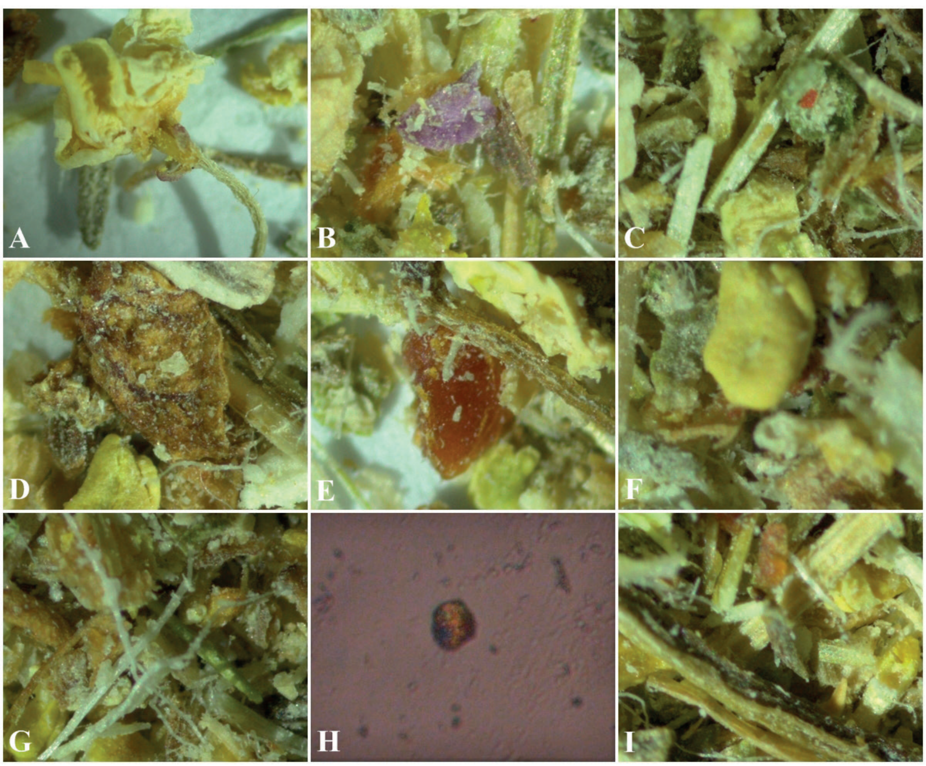

Fig. 3. Composition of herbal tea marked as Sample No. 2 intended for treatment of upper respiratory tract problems. **A**, flower fragment of *Sambuci flos*; **B**, flower fragment and **C**, stem fragment of *Thymi herba*; **D**, flower fragment of *Robinie flos*; **E-F**, fruit fragment of *Cynosbati fructus*; **G**, root fibers of *Althaeae radix*; **H**, pollen grain and **I**, flower fragment of *Chamomilae flos*.

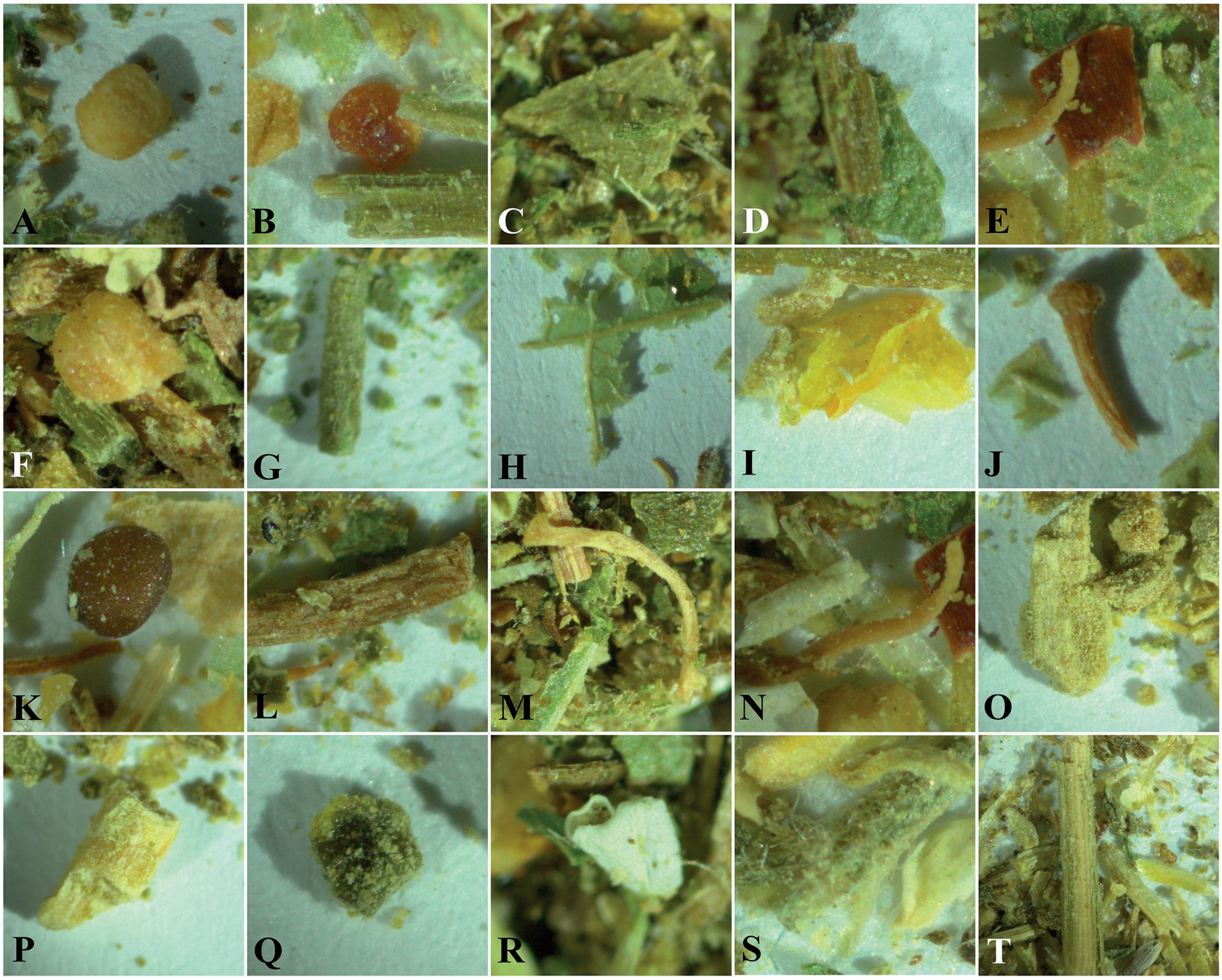

Fig. 4. Composition of herbal tea marked as Sample No. 3 intended for treatment of cardiovascular system problems. **A-B**, fruit fragments of *Cynosbati fructus*; **C-D**, petiole and leaf fragments of *Betulae folium*; **E**, fruit fragment, **F**, seed fragment and **G**, stem fragment of *Crategi fructus*; **H-J**, flower, bract, petiole, fragments of *Tiliae flos*; **K**, seed of *Tiliae flos*; **L-N**, root fragments of *Valerianae radix*; **O-Q**, stem and flower fragments of *Visci herba*; **R**, flower, **S**, stem and **T**, leaf fragments of *Milefolii herba*.

firmed in Sample No. 4 due to the presence of appropriate identification characters: a leaf fragment with calcium oxalate crystals (Fig. 5I) and a leaf stalk (Fig. 5J), coupled with the characteristic smell of lemon.

For Sample No. 5, the presence of *Lupuli strobili* was shown by detection of the inflorescence axis (Fig. 6A) and the bractea with glandular trichomes (Fig. 6B). The aboveground parts of *Calluna vulgaris* plant were detected in the tea mixture, which shows the presence of flowers and stems with leaves (Figs 6C, D). *Rubi fruticosi folium* was also found in Sample No. 5 due to the presence of leaf blade fragments (Fig. 6E). Whole fruits of hawthorn (*Crategi fructus*), as well as rose hips (*Rosae caninae fructus*) were present in this tea mixture (Figs 6L, M, N). Ground parts of blueberry leaves were present in the tea mixture (Figs 6I, J, K). The *Leonurae cardiacae herba* were found in the tea mixture, which is indicated by the presence of cut parts of the stem (Fig. 6F), leaves (Fig. 6H) and flowers (Fig. 6G). Fragments of bean pericarp were also found in the tea mixture (Fig. 5O).

Analysis of a tea mixture intended for use by consumers with type 2 diabetes, Sample No. 5 (Fig. 6), showed that it does not contain horsetail herb (*Equiseti herba*), lemon balm leaf (*Melissae folium*), dandelion root (*Taraxaci radix*) and yarrow herb (*Millefolii herba*), although these were specified on the product label. On contrary, some of the components determined in the tea mixture, such as hop cones (*Lupuli strobili*) and hawthorn fruit (*Crategi fructus*), were not listed on the declaration. Hop cones (*Lupuli strobili*) exhibit an

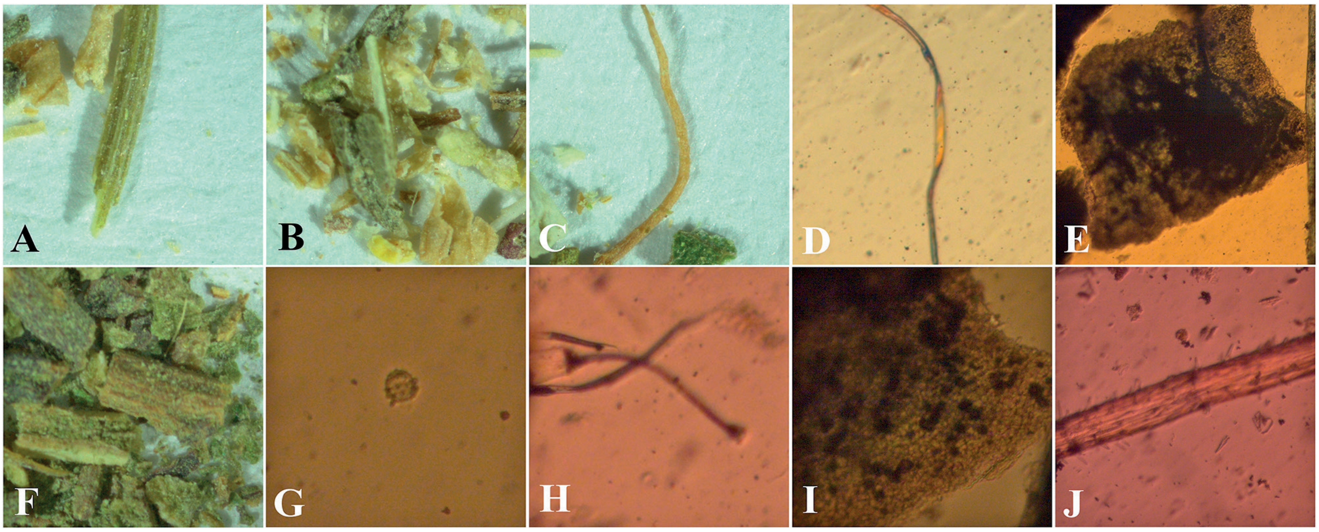

Fig. 5. Composition of herbal tea marked as Sample No. 4 intended for treatment of nervousness and anxiety. **A**, stem fragments and **B**, root fragment of *Milefolii herba*; **C**, root fragment of *Valerianae radix*; **D**, non-glandular trichome of *Folium cum flore crategi*; **E**, leaf fragment with glandular and non-glandular trichomes of *Menthae piperithae folium*; **F**, stem fragments of *Folium cum flore crategi*; **G**, pollen grain of *Chamomilae flos*; **H**, non-glandular trichomes of *Menthae piperithae folium*; **I**, leaf fragment with calcium-oxalate crystals and **J**, leaf petiole fragment of *Mellisae folium*.

Fig. 6. Composition of herbal tea marked as Sample No. 5 intended for consumers diagnosed with type 2 diabetes. **A**, axis of inflorescence and **B**, bracteum fragments with glandular trichomes of *Lupuli strobili*; **C**, stem fragments and **D**, flowers of *Callunae herba*; **E**, leaf fragment with non-glandular trichome of *Rubi fruticosi folium*; **F**, stem fragment, **G**, flower and **H**, leaf fragment of *Leonurae cardiacae herba*; **I-K**, leaf fragment, calcium-oxalate crystals in leaf fragments of *Myrtilli folium*; **L**, frut in *Crategi fructus*; **M-N**, fruit morphology in *Rosae caninae fructus*; **O**, pericarp fragments of *Phaseoli pericarpium*.

anxiolytic effect and do not help in mitigating symptoms of type 2 diabetes (WHO 2007c). Hawthorn fruits are used as coronary vasodilators, as well as traditional antihypertensives, without affecting blood sugar levels. Interestingly, only two components identified in the examined herbal mixture, blueberry leaves (*Myrtilli folium*) and bean fruit pericarp (*Phaseoli pericarpium*) have a demonstrated history of usage as antihyperglycemic agents (Helmstädter 2010; Helmstädter and Schuster 2010). This product contained specific adulterations not declared on the product's packing. Furthermore, the degree of impurities determined in this tea sample (10.4%) was five to ten times higher than levels proscribed by relevant documents (1 or 2%) Moreover, it was observed that the impurities are dominated by herbal fragments from species of the families Pinaceae and Poaceae. Contaminants from the Pinaceae family most likely belong to the leaves from genus *Pinus*, due to its resin ducts, wrinkled chlorenchyma and two conductive bundles in the center of the leaf (Fig. 7)

After evaluation of the investigated tea mixtures, it can be observed that four out of five evaluated samples meet the criteria proscribed by relevant documents. Namely, these samples contain all authentic plant drugs and none of the drugs found in the tea mixtures was adulterated. Moreover, all of the drugs listed on the declaration and found in the tea mixtures can be used for treating the medical conditions listed on the packaging of the tea sample.

DISCUSSION

As previously demonstrated, four tea mixtures completely matched the declared content in terms of herbal drug presence, since all herbal drugs listed on the declaration were found in the tea mixture. Analysis of the tea mixture in Sample No. 5 revealed a significant mismatch between the determined content and the content listed on the declaration. A possible cause for this disagreement in Sample No. 5 is that it does not originate from the same production process as the other four samples. Results show that four samples that originate from one company were produced in facilities which have implemented the required standards of International Organization for Standardization (ISO) and Hazard analysis and critical control points (HCCP).

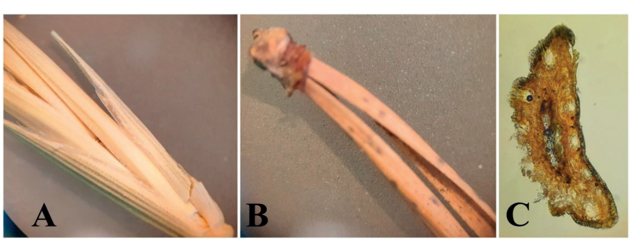

Fig. 7. Foreign matter analysis – characters necessary to identify the adulterations. **A**, Flower and leaf details of *Graminis herba*; **B**, Leaf detail of *Pini folium*; **C**, Leaf cross section of *Pini folium*.

The foreign material identified in Sample No. 5 consists of plant species without declared pharmacological effects. The possible reasons for their addition to the mixture is to increase the weight of the herbal product or the initial low quality of herbal materials used for mixture production. The degree of foreign impurities can be determined by method presented in this paper, but only in samples that do not display a high degree of fragmentation. In samples prepared with a high degree of grinding, it is impossible to physically separate certain parts (including those of foreign origin) with tweezers and other instruments. That is why only Sample No. 5 was subjected to this type of quality control, while the other samples were not analyzed. This is one of the main limitations of this method. Additionally, it prevents quantification of foreign matter in these types of samples.

All tea mixtures except Sample No. 5 met the requirements of the consumer. In the tea mixture present in Sample No 5., plants that do not have the biological effect stated on the packaging were identified.

The process of herbal material identification allows us to utilize various macroscopic and microscopic characters and easily confirm the identity of plant species which are integral parts of the tea mixture. The characters used to identify plants can be found in all parts of plants (both meristematic and permanent tissues, characters present on flowers, fruits and seeds, as well as vegetative organs) which was confirmed during this and some previous studies (Au et al. 2009, 2012; Serrano et al. 2010; Zhao et al. 2011).

In the case of this specific sample, macroscopic characteristics were used more frequently than microscopic ones. This can be explained by the fact that in order to identify most of the plant material in these samples of tea mixtures, it was enough to find macroscopic characteristics that confirm the identity of the biological source of the drug. These characteristics provided enough information about the biological origin of the raw material, so that microscopic methods (which require more complex sample preparation) did not have to be used.

Light microscopy is the method of choice for these analyses, since it enables fast authentication of the content of tea mixtures, as confirmed during this study, and stated in all guidelines of the authorities dealing with this topic (Ph. Jug. IV 1984; WHO 1998; Ph. Eur. 8.0 2013). Other methods based on identification of chemical and molecular-biological characters, such as polymerase chain reaction (PCR), high performance liquid chromatography (HPLC), or gas chromatography-mass spectrometry (GC-MS) can also be used for authentication of compounds present in tea mixtures (Ichim et al. 2020). However, these methods require a significant amount of time and resources, which is not the case with the method presented in this paper. Although chemical methods are very precise for identification, this does not mean that light microscopy methods are of significantly lower resolution and that only chemical methods should be used. The reduced possibility of error is supported by the fact that each character photographed in a tea mixture used in this paper is compared to a character on plant matter of known origin.

Furthermore, light microscopy can be used for identification of raw plant material because it provides results of acceptable quality, even in the case of ground herbal material. The method is sufficiently reliable and economical in comparison with other methods that are used to define the biological sources of herbal drugs and rely on deoxyribonucleic acid (DNA) technology (Serrano et al. 2010; Ichim et al. 2020).

CONCLUSION

The results from the present study show that botany expertise is important for quality control of herbal teas mixtures, and it has been proven that macro- and micromorphological characteristics represent an important resource for quality control of these tea mixtures. Five samples of tea blends used in this study were subjected to quality control in order to identify plant material, and the whole process is sufficiently precise, reliable, robust and economically viable. Four of the five samples did not contain adulterations and can be used to treat the conditions stated on their declarations. Application of the methods used in this study as identifiers facilitated authentication of herbal tea component, regardless of the degree of grinding of the analyzed sample. Microscopic and macroscopic morpho-anatomical characteristics can also be effectively applied for determination of impurities and adulterations in tea mixtures, which is shown in the example of the fifth tea mixture in which the presence of adulterations was confirmed with great certainty.

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