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Original paper

Morphological characterization of cinnamon bark and powder available in the Serbian market

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Summary: *In vitro* and *in vivo* research suggests that cinnamon, which is widely used as a spice, could have significant medicinal potential. However, the problem of using this spice for medicinal purposes is that there are hundreds of species and subspecies of cinnamon plant, and although the official source for spice production is *Cinnamomum verum* (*zeylanicum*) – Ceylon cinnamon; *C. cassia* – Chinese cinnamon, and *C. burmanii* – Indonesian cinnamon are commonly found on the market. In powder form it is practically impossible to distinguish between these types of cinnamon without a microscope or analytical methods. The aim of the present study is the analysis of cinnamon on the Serbian market, including verification of its origin using macroscopic and microscopic methods. Bark samples were observed under a binocular magnifier ZEISS STEMI 508 with AXIOCAM ERc 5s camera to determine fine differences between species. The powder was examined under an AXIOLAB A.1 binocular microscope with AXIOCAM ERc 5s camera. During macroscopic identification, visual differences were clearly detected and results clearly showed that in toto bark of these three species of cinnamon can be differentiated even if the type and origin are not designated on the package. During microscopic observation of powdered samples, *Cinnamomum burmanii* was the easiest for differentiation because of the existence of authentic plates of calcium oxalate. However, more specific characters are needed in order to distinguish between Ceylon and Chinese cinnamon powder samples. One of the possible ways to overcome this problem may be through the measurement of fiber diameters where significant differences were found. Based on the obtained results it can be concluded that microscopic techniques, which are affordable, may be a useful tool for elucidation of the differences between the most commonly present cinnamon species on the market.

Keywords: cinnamon, microscopic techniques, quality control, safety.

INTRODUCTION

The bark of various cinnamon species is an important spice that is used all over the world. This aromatic plant is not only used for cooking but also in traditional medicine (Rao and Gan 2014). The genus *Cinnamomum* has three main species that are used to produce bark and powder that can be found on the market (Ravindran et al. 2003). Ceylon or Sri Lankan cinnamon (*Cinnamomum verum* J. Prese syn. *C. zeylanicum* Blume; Lauraceae) is considered to be the official source of the drug (*Cinnamomi cortex*) according to the pharmacopeia (European Pharmacopoeia 6.0 2008). *Cinnamomum cassia* J. Presl (Lauraceae) is the second important species of cinnamon and it is used more often because of its economic availability. However, Indonesian cassia (*C. burmanii* Blume; Lauraceae) can also be found on the market. This is now the most commonly imported cinnamon, and has replaced the more expensive Ceylon cinnamon in Canada, United States and Europe (Wang et al. 2013). One important difference between these species is their coumarin content (Woehrlin et al. 2010). Coumarins have a strong anticoagulant effect and can express potential hepatotoxicity. The coumarin content in Ceylon cinnamon is insignificant and it is not known if such a small coumarin content can cause unfavorable health effects, whereas the coumarin concentration in *C. cassia* is much higher and it can cause health risks if it is consumed very frequently in larger quantities. Similarly to cassia, Indonesian cinnamon may contain significant amounts of coumarin. As a result of certain findings, a few countries have restricted the regular usage of *C. cassia* (Lungarini et al. 2008). Also, some investigations suggest that coumarin content varies widely. The amount of coumarin

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differs even within the same tree. Reported levels have been from 486 mg/kg in Ceylon cinnamon to up to 10900 mg/kg in cassia cinnamon (Woehrlin et al. 2010). It was first thought that coumarin has genotoxic and carcinogenic effects. The following research has suggested that coumarin is not genotoxic, but hepatotoxic and anticolagulant effects are responsible for the increased health risk. Therefore, in 2004, the European Food Safety Authority (EFSA) had designated a value of 0.1 mg per kg body weight for tolerable daily intake (TDI) according to data about hepatotoxicity on animals (Sproll et al. 2008). In Serbia, all three species of cinnamon are available in dried form, both as *Cinnamomi cortex in toto* and *Cinnamomi pulvis*, but Chinese and Indonesian cassia are more affordable. In powder form, it is practically impossible to distinguish between these types of cinnamon, without a microscope or analytical methods. Unlike *Cinnamomi pulvis*, the differences between the bark of these three species can be distinguished macroscopically, but the average consumer knows little about these differences, especially when the biological source of the drug is not clearly listed on the declaration (Woehrlin et al. 2010).

Cinnamon has high medicinal potential and a wide range of possible positive effects on the human body. Clinical studies have shown that cinnamon supplements can improve glycemic indices and lipid profiles of patients with type II diabetes. Cinnamon exhibits a hypoglycemic effect by various mechanisms. It is considered that cinnamon extracts activate insulin receptor kinase and inhibit insulin receptor dephosphorylation, which is important to insulin sensitivity. Furthermore, cinnamon can inhibit glycogen synthase kinase-3 that causes an increase in glucose uptake. Some researchers have found that cinnamon can play a role in intestinal glucose absorption by inhibiting α-amylase and α-glucosidase. All of these complex mechanisms also affect weight loss and may increase HDL cholesterol and insulin. This creates possibilities for using cinnamon as supportive therapy in metabolic syndrome (Costello et al. 2016; Zare et al. 2018). It is important to note that it is necessary to supply at least 1.5 grams of cinnamon for 12 weeks in order to achieve beneficial effects on health (Askari et al. 2013). This long-term use involves the inclusion of large amounts of cinnamon, therefore it is important to use Ceylon cinnamon with a low coumarin content to avoid potential health hazards (Lungarini et al. 2008).

Apart from the influence of cinnamon on metabolism, a number of authors have suggested that cinnamon essential oil has high antimicrobial potential and can be used as an alternative remedy for synthetic antimicrobial agents (Shabani et al. 2016; Vasconcelos et al. 2018).

All this supports the wide use of cinnamon both as a spice as well as a therapeutic agent. For this reason, correct identification and quality analysis are very important in order to contribute to the safety of its use. Cinnamon is not a native plant for Serbia and consequently it is imported. Before being released into the market, the drug must meet the criteria prescribed by the rules on spice quality (Pravilnik o kvalitetu začina, ekstrakata začina i mešavina začina 2014). The Rulebook defines the minimum content of essential oil and maximum moisture and ash but there is a need for more rigorous control of origin, quality and safety of imported cinnamon. The quality control is significant especially as a part of the fight against Food Fraud. These factors are of great importance for maintaining a high level of food safety (Spink and Moyer 2011; Charlebois et al. 2016).

The aim of the present study was analysis of cinnamon available in the Serbian market along with verification of its origin through macroscopic and microscopic methods.

METHOD AND DESIGN

All five samples of cinnamon (bark and powder) were purchased in local grocery stores and health food stores in Novi Sad and Belgrade (Table 1). Bark samples were observed under ZEISS STEMI 508 stereo microscope with AXIOCAM ERc 5s camera, to determine fine differences between the species. The powder was examined under an AXIOLAB A.1 binocular microscope with AXIOCAM ERc 5s camera using *general reagent* (Tucakov 1996). Images were taken using an AXIOCAM ERc5 (Zeiss) digital color camera. The measurements of fiber diameters were conducted in the program AxioVision 4.9.1. The following measurements were taken for 31 fibers from each sample: Ceylon and Chinese cinnamon. After testing for normality of the

Tabel 1. Cinnamon samples used for morphological characterization.

Samples	Form	Country of origin	Source	Date of collection/ production	Best before
1	Powder	Indonesia	not specified	01. 2018.	31. 01. 2021.
2	Powder	Sri Lanka	*C. zeylanicum*; Laureaceae	not specified	12. 05. 2019.
3	Bark	Sri Lanka	*C. zeylanicum;* Laureaceae	not specified	10. 05. 2019.
4	Bark	Indonesia	not specified	not specified	31. 03. 2019.
5	Bark	China	not specified	not specified	03. 11. 2018.

distribution (Kolmogorov-Smirnov test), statistical analysis was performed using a *t*-test for independent samples in the program IBM SPSS Statistics 20. Values $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The enormous herb and spice industry is constantly growing, yet only 2% of the world's herbs and spices are produced in Europe while the rest is imported from other continents. Due to the distance between producer to consumer, opportunities for adulterations of herbs and spices may occur at any point along the long and complex supply chains. Consequently, there is a need for rapid screening techniques in order to detect and help prevent fraud from occurring in the industry (Galvin-King et al. 2018). In view of the widespread use of cinnamon as a spice and ingredient of dietary supplements on the one hand and difficulties in cinammon identification on the other, there is a risk of misuse of identification facts with the aim of deliberate fraud in the market for the achievement of economic benefits. This situation is defined as Food Fraud and it violates consumer safety due to the different content of hepatotoxic coumarin in these three types of cinnamon (Woehrlin et al. 2010).

In the present study, during the macroscopic identification of cinnamon in the stick, visual differences were clearly detected. Cassia sticks possess a hard and thick layer of bark rolled up in one stick, while Ceylon is composed of a few soft and thin bark layers rolled up in one stick that look like a cigar. Indonesian cassia is similar to Chinese cassia with one layer of bark rolled up in the stick. Also, it was clearly visible that the investigated bark of Chinese cinnamon was stored for a long period with deposits of mildew present on its surface (Fig. 1).

Based on what was observed and according to the literature data, it is clear that *in toto* bark of these three species of cinnamon can be distinguished even if the type and origin are not designated on the package (Shylaja and Manilal 1992; Ravindran et al. 2003; Woehrlin et al. 2010).

In the case of cinnamon powder it is practically impossible to distinguish between these three types of cinnamon. For this reason, microscopic identification is needed to differentiate between these species. According to the literature data, there are some differences in the microscopy of these types of cinnamon that depend on many factors. The presence of certain anatomical structures depends on the region of the stem which is used for producing *Cinnamomi cortex*. Additionally, Sri Lankan cinnamon has four commercial grades (guills, guillings, featherings and chips) (Ravindarn et al. 2003). All these factors make it difficult to identify the drug in the powder.

During microscopic observation of powder samples, *C. burmanii* was the easiest to distinguish because of authentic plates of calcium oxalate (Fig. 2). The powder of the Ceylon cinnamon was characterized by a number of rounded

Fig. 1. Drug in sticks (Chinese, Indonesian and Ceylon cinnamon sticks, respectively) and deposit of mildew on Chinese cinnamon bark.

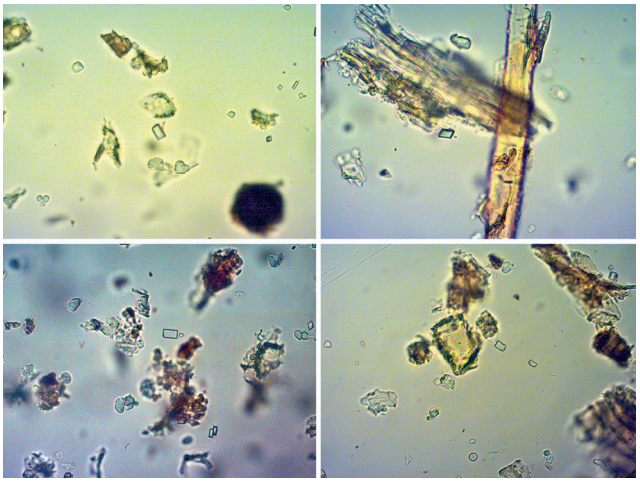

Fig. 2. Plates of calcium oxalate in *Cinnamomun burmanii*.

sclereides, present singly or in small groups, abundant fibers with small uneven lumen and a lot of starch grains. Something that was very noticeable was that cork fragments were absent or very rare. Also, significant microscopic characters were needle-shaped crystals of calcium oxalate but they were very rare and traceable (Fig. 3). The main characteristics of *C. cassia* powder were a large amount of starch granules and greater fiber diameter (Fig. 4). The observed characteristics of all three types of cinnamon were in accordance with the literature data (Ravindarn et al. 2003). However, more specific characters are needed in order to distinguish Ceylon and Chinese cinnamon powder samples. One of the possible ways to overcome this problem may be through the measurement of fiber diameters, where significant differences were found (Fig. 5A, B). The average diameter of fibers in Ceylon cinnamon was 52.00 ± 5.25 μm while in Chinese cinnamon it was 69.60 ± 8.85 μm. *T*-test for independent samples showed that there was a statistically significant difference in diameters between the fibers of these two cinnamon species (with $P = 0.001$). These findings indicate that the diameter of fibers present in Ceylon and Chinese cinnamon powder could be used for elucidation of the origin of these spices present on the market.

CONCLUSIONS

Cinnamon is widely used as a spice with potential health benefits. For this reason, there is a need for all stages of its supply chain to undergo stricter monitoring in order to avoid potential health risks. Microscopic techniques, being one of the most affordable, may be a useful tool for the elucidation of differences between the most commonly present cinnamon species on the market.

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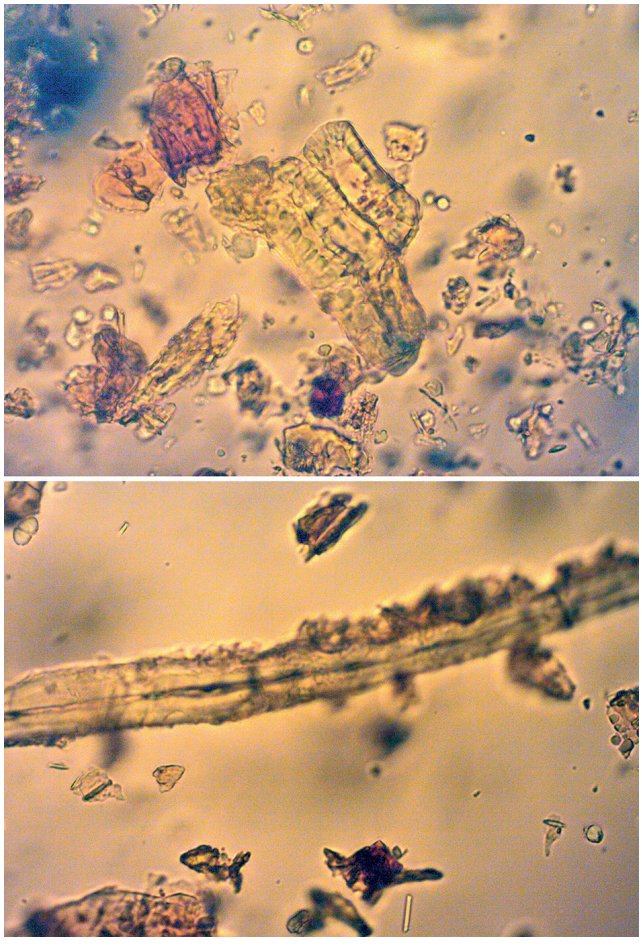

Fig. 3. Sclereides and needle-shaped crystals of calcium oxalate in *Cinnamomum zeylanicum*.

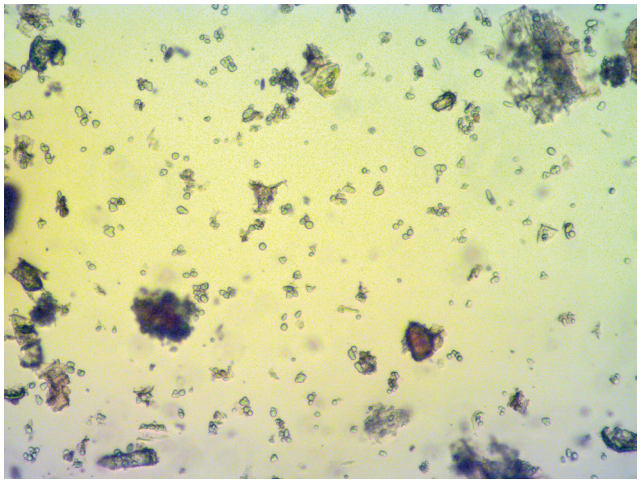

Fig. 4. A large amount of starch granules in *Cinnamomum cassia*.

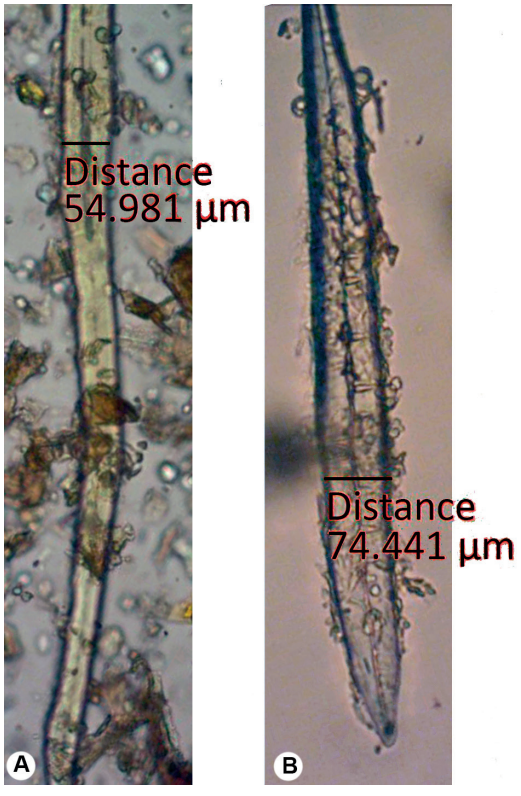

Fig. 5. Fiber with uneven lumen in *Cinnamomum zeylanicum* (**A**); Fiber in *Cinnamomum cassia* (**B**).

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