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Research Article

Contribution to the microscopic identification of Zingiberis rhizoma

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Abstract

Zingiber officinale Roscoe has a very long history of use throughout the world, both as a spice and as a medicinal plant. During the last two years in the midst of a global pandemic of SARS-CoV-2 the use of various herbal products with ginger rhizome as active ingredient increased because of its numerous health beneficial properties. A detailed characteristic of the crude powdered drug is presented by microscopic photographs for the first time. Although the structure of ginger rhizome is relatively simple and well-studied, the presented results in combination with the detailed analysis of the literature provide additional insight to the pharmacopoeial guidelines for the identification of ginger powder.

Keywords

ginger, crude powder, microscopy

Introduction

One of the most accessible and popular sources of natural antioxidants and other health promoting compounds are plants that combine the qualities of food, spices and traditional remedies. Ginger is one of the most prominent representatives of this group of plants, which is cultivated intensively and globally used. Over the last two decades, the interest in the properties of this plant is constantly increasing. As a confirmation of this can be considered the big increase in the world production of ginger of 153% for the period 2007–2019. In 2019, the amount of ginger produced worldwide was 4,081,374 tonnes in big contrast with the 1,608,984 tonnes for 2007 (FAOSTAT 2021). During the last two years in the midst of a global pandemic of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) the use of various herbal products with ginger rhizome as active ingredient increased even more because of its pronounced antiviral properties (Jafarzadeh et al. 2021). Currently ginger is referred as promising herbal medicine useful to relieve symptoms related to upper respiratory conditions, pulmonary fibrosis, pneumonia, ARDS (Acute respiratory distress syndrome) through its anti-inflammatory effect and immune response improvement. Based on clinical and safety studies it is assessed as a potential adjuvant therapy to COVID-19 (Boozari and Hosseinzadeh 2020; Silveira et al. 2020; Thota et al. 2020).

Zingiber officinale (ginger) has a 2000 years history of use as a traditional medicinal plant in Chinese, Ayurvedic and Tibb-Unani herbal medicines (Ghosh et al. 2011). It is also used as spice, natural additive and flavoring in a wide variety of products, including sweets such as cakes and biscuits, and also in beverages such as ginger ale and ginger wine. It is one of the main ingredients of curry powder. *Z. officinale* is officially included in Ayurvedic, Indian herbal, Chinese, Japanese, African, American, British

and European Pharmacopoeias. Modern research report a great number of health properties such as antiemetic, anti-inflammatory, antioxidant, antithrombotic, hypolipidemic and hypoglycaemic, cardiovascular, antineoplastic, anti-infectious, thermogenic, analgesic, nephro-, neuro- and hepatoprotective, etc. (EMA/HMPC 2010; Ghosh et al. 2011).

A large-scale review study on the botanical authentication through chemical methods of herbal products sold over six continents shows that still 27% of the products proved to be adulterated (Ichim and Booker 2021). The use of classical microscopic methods for the identification of plant substances can help reduce this percentage. The inclusion of these methods in the quality control of herbal products at the stage of primary processing of plant substances is a good prerequisite for obtaining high quality products. Despite the great interest in the phytochemical composition and pharmacological effect of rhizome extracts from *Zingiber officinale* Roscoe, we found not so many studies related to its anatomy and microscopic characteristics of the powder. They focus on the general anatomy of the rhizomes and microscopic characteristics of powder (Remashree et al. 1997; WHO 1999; Ravindran et al. 2005; European Pharmacopoeia 2014; Rungsung et al. 2014; Uma and Muthukumar 2014; Pawar et al. 2015; Kala et al. 2016) and the secretory structures (Mu et al. 2015; Indriyani 2017; Liu et al. 2020). The microscopic characters of the powdered rhizome of *Z. officinale* have been described in a few sources (Jackson and Snowden 1990; WHO 1999; Ravindran et al. 2005; European Pharmacopoeia 2014) but accompanied by limited amount of visual material that does not include any microphotographs.

The aim of this study was to give detailed microscopic characteristic of the powdered *Zingiberis* rhizoma through microphotographies which can be useful in the authentication of this herbal substance.

Materials and methods

The ginger rhizomes were procured from the local market in Bulgaria, dried in a drying oven at 35 °C and grounded with pestle in porcelain mortar to powder. The coarse powder was reduced through a number 355 sieve following the classical pharmacopoeial method for microscopic examination of powdered herbal drugs (Ph. Eur. 8.0). Chloral hydrate was used as a clearing solution prior to mounting. Thirty temporary microscopic slides were mounted using the fine fraction of the ginger powder and glycerine as media. For comparison of the results, several longitudinal sections (LS) of a fresh rhizome cut by hand with a blade were made. Some sections were stained with a mixture of 10 ml 70% ethanol and 5 drops 0.1% Safranin O solution for 1 hour.

Qualitative microscopic analysis of the crude powder of ginger was performed using standard methods (Trease and Evans 2002; WHO 2011; European Pharmacopoeia 2014).

Results and discussion

Despite the evolving high-tech methods for the identification of plant substances based on DNA-sequencing, chromatography, microscopic image processing through probabilistic neural network (PNN) algorithms, etc. (Chavan et al. 2008; Andayani et al. 2020), classical microscopic examination according to pharmacopoeial principles remains the most cost-effective approach not only for research but also for the needs of botanical industries in case the diagnostic microscopic characteristics are preserved. That is because it is fast, cheap and reliable. On Figures 1–8 are presented microphotographs of all characteristic features of powdered ginger rhizome following European Pharmacopoeia 8.0. From their detailed examination it became clear that the presented diagnostic elements of the powdered ginger appear with different frequency in the microscopic slides. In all samples the most abundant objects were various in size parenchymal fragments with thin-walled, isodiametric cells (Fig. 1) and numerous starch grains outside these cells (Fig. 2). The parenchymal ground tissue rich in starch appears to be one of the most abundant tissues in the rhizomes of ginger. It forms the best part of the cortex and a significant part of the stele (Ravindran et al. 2005; Kala et al. 2016).

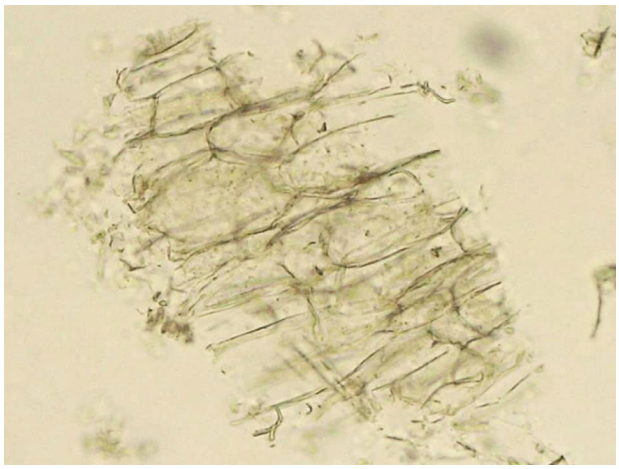

Figure 1. Fragment of parenchymal cells.

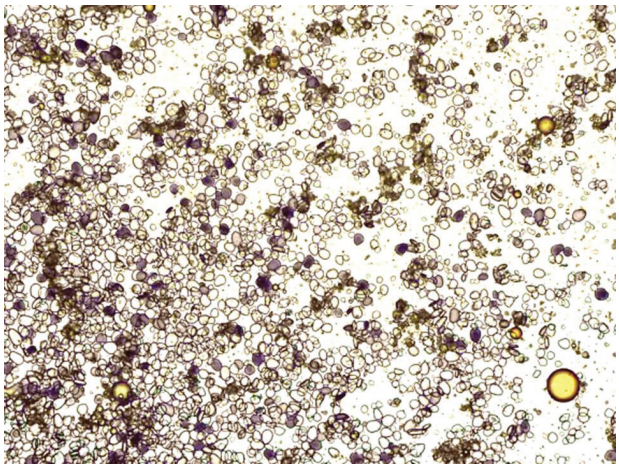

Figure 2. Starch grains.

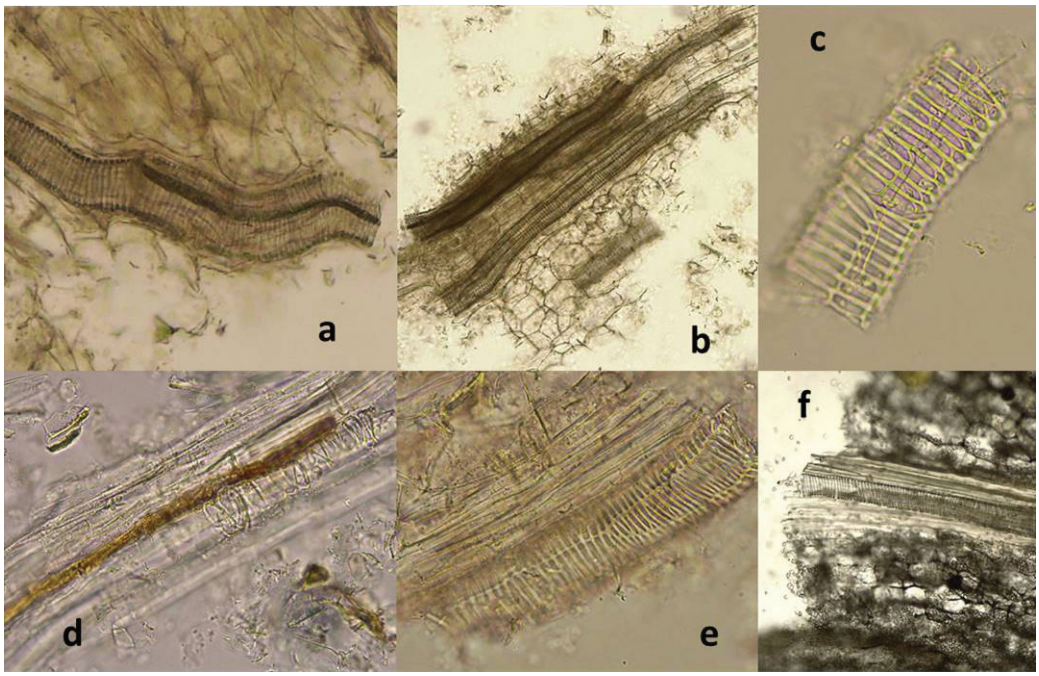

Figure 3. Fragments of xylem elements and related structures: **a, b, c** scalariform xylem elements; **d** narrow cells with brown pigment accompanying the xylem elements; **e** scalariform perforation plate of xylem vessel; **f** fragment with helical xylem elements.

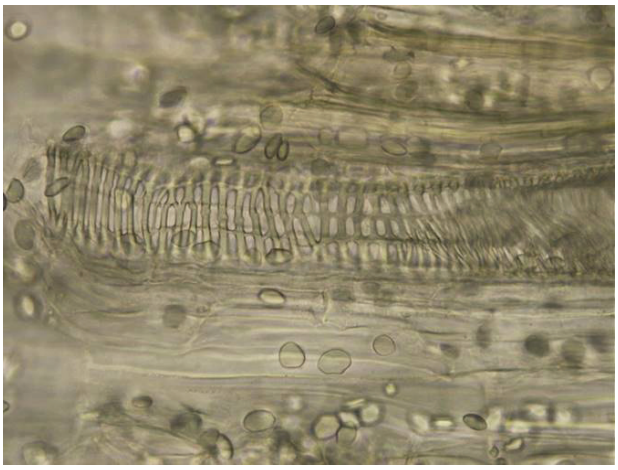

Figure 4. Fragment of reticulate xylem vessel.

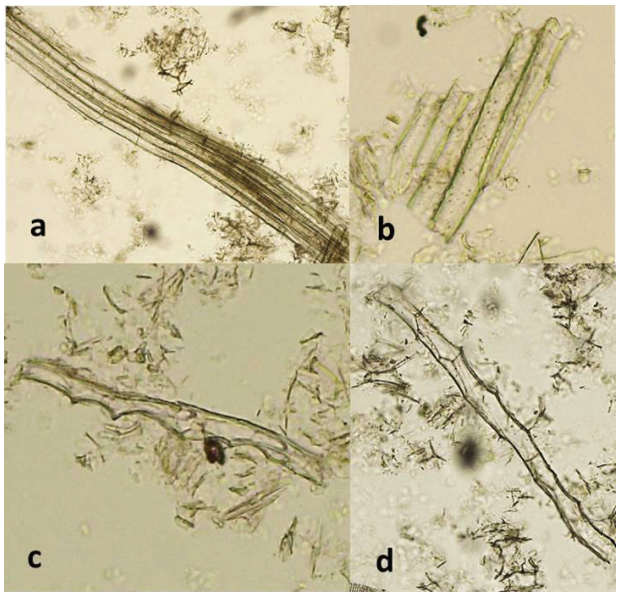

Figure 5. Fragments of sclerenchymatous fibres: **a** fragment of bundle sheath; **b** fragment of sclerenchymatous fibres; **c, d** sclerenchimatous fibres with dentate walls.

Next in frequency were observed fragments of xylem elements with scalariform secondary cell-wall thickenings (Fig. 3a–c), scalariform perforation plates, (Fig. 3e) and sclerenchymatous fibres (Fig. 5). According to the literature reference, most authors report that the most common xylem elements are scalariform but they do not agree on whether these elements are highly lignified (Liu et al. 2020), poorly lignified (Jackson and Snowden 1990) or unlignified (Remashree et al. 1997; Pawar et al. 2015). We observed a significant reaction for lignin to the solution of Safranin O of both the xylem elements and the mechanical fibres (Fig. 6). Ravindran et al. (2005) distinguished the ginger xylem elements of mainly tracheids with scalariform or helical thickenings and rarely vessels with scalariform to reticulate thickenings. The same authors noted that vessels occurred only in *Z. officinale* in comparison to other closely related species as *Z. roseum*, *Z. zerumbet* and *Z. macrostachyum*. These species specific vessels with reticulate thickenings are relatively rare because they originate only from the bundle zone adjacent to the pericycle (Ravindran et al. 2005). We found many of these vessels in the LS of bundles located inward from the pericycle adjacent to it (Fig. 4) and only few of them in the ginger powder samples. A possible reason for this may be that the source material of the dried rhizome was not sufficiently grounded with the mortar. In that case probably a significant part of the large and best developed conductive bundles with strong sclerenchymatous sheath,

Figure 6. Stained bundle sheath with xylem elements.

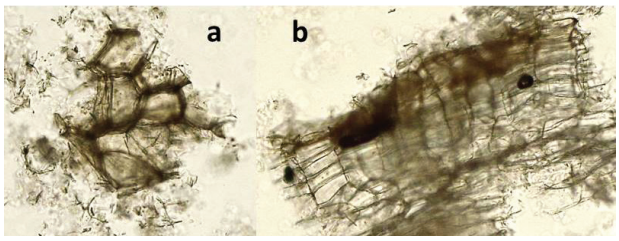

Figure 7. Cork cells: **a** longitudinal view; **b** transverse view.

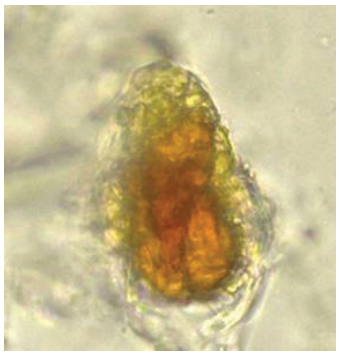

Figure 8. Oleoreisin cell.

which include the characteristic for *Z. officinale* reticulate xylem elements do not pass through a number 355 sieve. Most of the xylem elements that we observed, regardless of the type of their secondary cell-wall thickening – helical, scalariform or reticulate (Figs 3, 4), were between 10 and 20 μm in diameter. Particularly for the reticulate vessels in our samples, these sizes are significantly smaller than the vessel diameter range 21–66 μm according to Ravindran et al. (2005). Possible reasons for this may be the different size and development degree of the sampled rhizome branches, as well as anatomical variations between the numerous subspecies and varieties of *Z. officinale* which are marketed today worldwide (Ashraf et al. 2014; Nayak et al. 2014).

The fragments of cork cells in longitudinal (Fig. 7a) and transverse (Fig. 7b) view are very rare and appear only in a couple of slides. These fragments are part of the phellem layer which was poorly presented in the investigated ginger powder samples. According to Ravindran et al. (2005) it develops after the harvest of the rhizome, during the storage period.

From all 30 microscopic slides of powdered ginger that we studied only on one was found well distinguishable fragment, containing small vessels and the accompanying narrow cells with brown pigment (Fig. 3d). Significantly more often these pigment cells can be observed in LS of fresh ginger rhizome. The same goes for the oleoreisin cells – we found only one sufficiently distinguishable such cell on the microscopic slides with powder samples (Fig. 8). According to Liu et al. (2020) the amount of starch grains and oil secretory structures are inversely related as in mature rhizomes the amount of starch decreases at the expense of oil cells and cavities. All these results should be taken into account in the microscopic identification of ginger powder as part of the quality control of this herbal substance.

Conclusions

Although the structure of ginger rhizome is relatively simple and well-studied, the presented study provides for the first time a complete description of the individual microcharacteristics of the powdered Zingiberis rhizoma through microphotographs, establishes their specific frequencies of occurrence, clarifies the diameter range of the xylem elements and confirms the degree of lignification of the xylem and mechanical elements by histochemical test. All this gives additional insight to the pharmacopoeial guidelines for identification of ginger powder and contributes to the efficiency of the microscopic analysis of Zingiberis rhizoma.

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