

Supplementary data file

Microscopic analysis of spice powders

1. Introduction

Powder microscopy is an evaluation/quality control method, used for medicinal plants to study the specific microscopic characters using different staining reagent. The detection of adulterants in a sample by performing a comparison study using authenticated sample.

2. Materials and methods (Ref 1 & 2)

2.1 Procurement and preparation of spice powder

Healthy growing spices of *Alpinia galanga*, *Cinnamomum zeylanicum*, *Foeniculum vulgare*, *Trigonella foenum-graecum* and *Myristica fragrans* were collected. The collected spices were shade dried, coarsely powdered with the help of pulverizer and used for microscopy studies.

2.2 Spice powder microscopy

The powdered spices were soaked in 20% Nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked spice powders with safranin and observed under the microscope, and the images were captured (Magnus MLXi-TR plus LED Trinocular microscope).

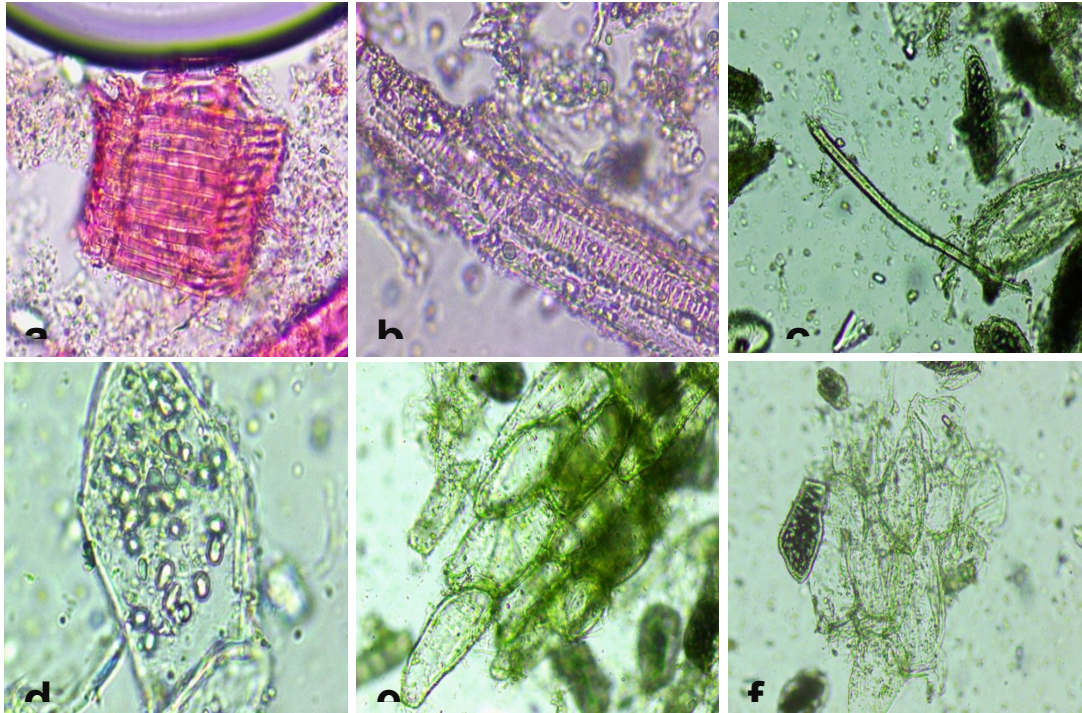
3. Observations and Results (Ref 3 & 4)

Generally, the powder microscopy study reveals that the spice powder contains many square and prismatic shaped calcium oxalate crystals. The powder also shows the presence of well-arranged pitted, annular and spiral vessels, fibres, starch granules, parenchyma, trichome, sclereids, oil globules, epicarp, endocarp, endosperm, and perisperm. For the studied spice samples, the microscopy images were presented in the below figures.

1. *Alpinia galanga* :

The diagnostic characteristics are,

- Abundant starch granules, which are simple and spherical or compound and most of them were round or oval-shaped.
- Parenchyma cells at places stuffed with an aggregation of small silica crystalline matter.
- Other identified characteristic features are vessels, fibres, and trichomes.



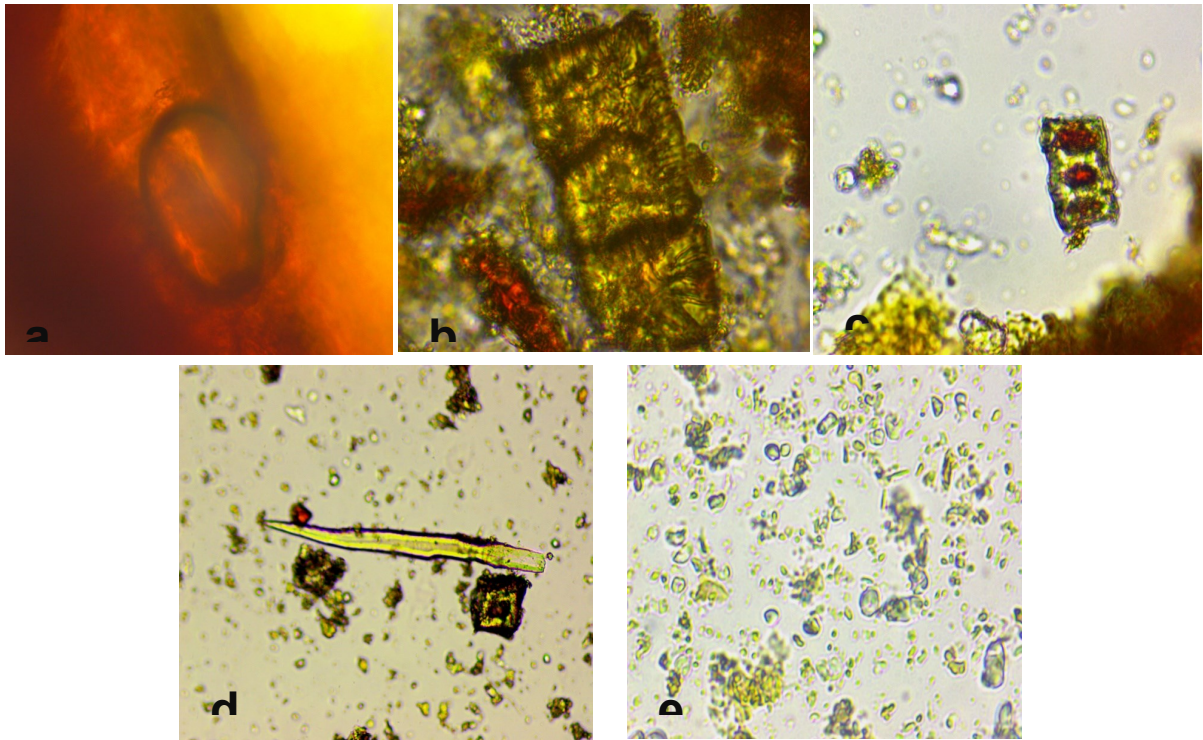
Supplementary Fig. S1a Microscopic images of *Alpinia galanga* spice powder Broad vessel associated with pitted fibers (a), Vessels and fibers (b), Simple trichome (c), Starch grains (d), Parenchyma crystalline matter (e), Parenchyma studded with silica (f).

2. *Cinnamomum zeylanicum* :

A reddish-brown powder with a characteristics of pleasant and aromatic odour and taste.

The diagnostic characteristics are,

- The abundant sclereids, which occur singly or, more frequently, in small groups.
- The abundant starch granules, which are small, simple and compound with up to four or more components.
- The thin-walled oil cells were found.
- Other identification characteristics are vessels, fibres.

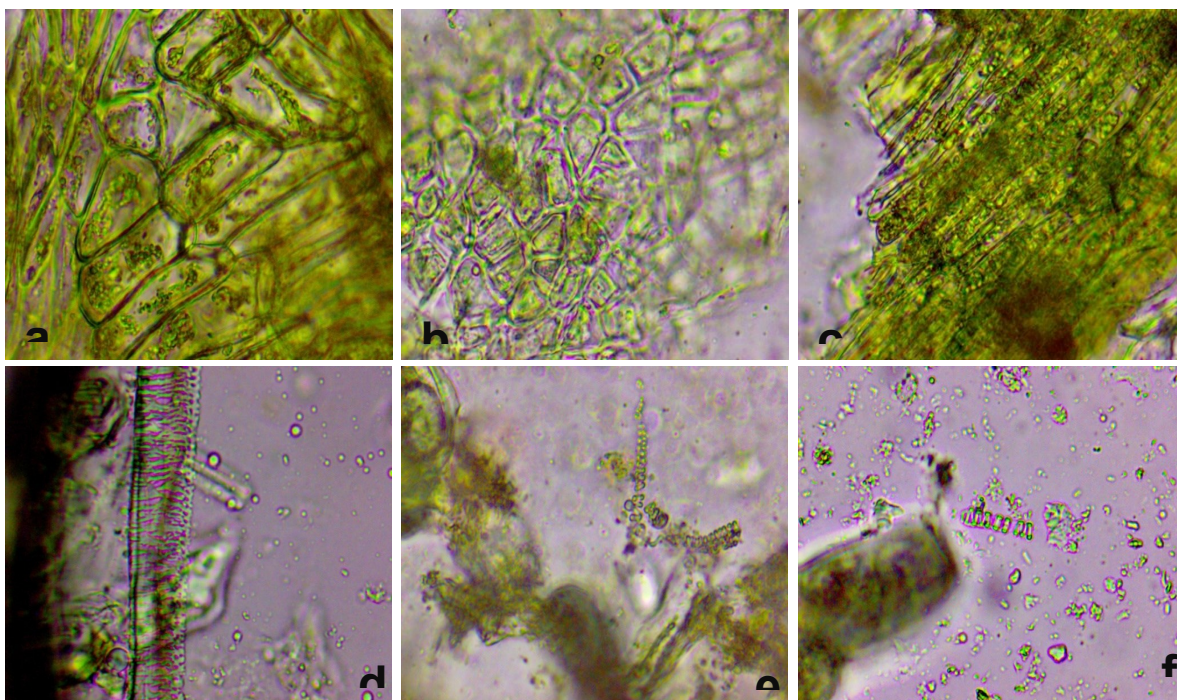


Supplementary Fig. S1b Microscopic images of *Cinnamomum zeylancium* spice powder
Single oil globule (a), Single sclereid (b), Sclereids (c), Fiber (d), Starch granules (e).

3. *Foeniculum vulgare* :

A yellowish-brown to greenish-brown powder with a pleasant, aromatic odour.

- The epicarp composed of a layer of colourless, thin-walled cells, polygonal in surface view.
- The endocarp composed of a layer of thin-walled, lignified cells, elongated in surface view and arranged in groups of about six or more cells with their long axes parallel to one another.
- The abundant endosperm composed of moderately thick-walled cells containing microcassette crystals of calcium oxalate.
- The fragments of lignified fibro-vascular tissue composed of small fibres, vessels and occasional larger vessels with reticulate thickening.

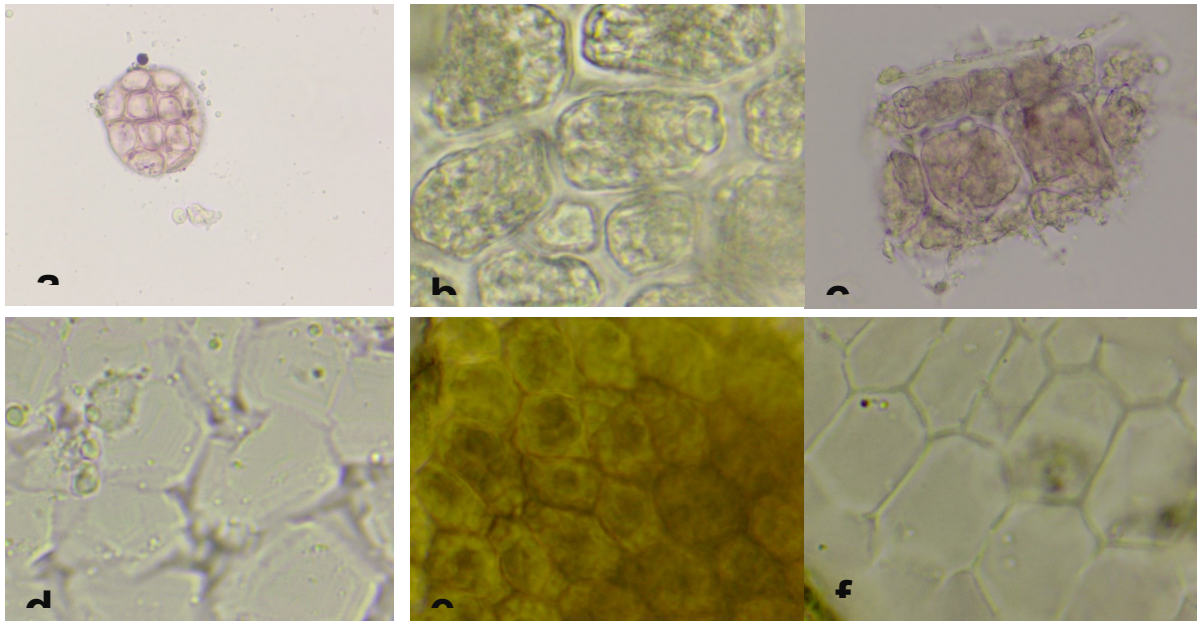


Supplementary Fig. S1c Microscopic images of *Foeniculum vulgare* spice powder. Endosperm containing microrosette crystals of calcium oxalates (a), Epicarp in surface view (b), Endocarp (c), Elements from fibro-vascular tissue (d), Elements from fibro-vascular tissue vessel (e), Fragment of reticulately thickened (f).

4 *Trigonella foenum-graecum* :

A pale-yellowish brown powder with a characteristic spicy odour and bitter and unpleasant in taste.

- The abundant parenchyma of cotyledons composed of thin-walled cells, some which are polygonal or undifferentiated or rounded.
- The fragment of the hypodermis of testa composed of a layer of cells with a characteristic appearance. In surface view, if viewed from, the rounded outline of the upper wall is seen and when viewed from below the polygonal outline is apparent.
- The epidermis of the testa when viewed from above, the cells are polygonal and a small lumen from which radiate distinct pits.

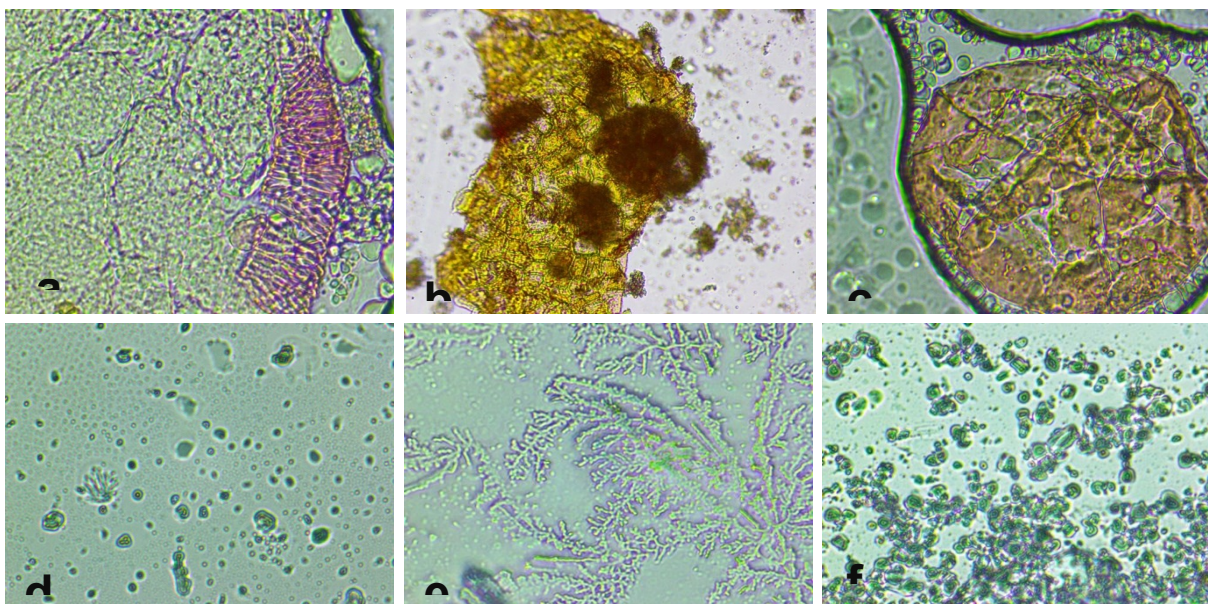


Supplementary Fig. S1d Microscopic images of *Trigonella foenum-graecum* spice powder Hypodermis of the testa in surface view from below (a), Hypodermis of the testa in surface view from above (b), Epidermis and parenchymatous cells of the cotyledons in sectional view (c), Layer of parenchyma (d), Epidermis of the testa in surface view, from above (e), Parenchymous layers (f).

5 *Myristica fragrans* :

A brown powder with characteristics, aromatic odour and an aromatic, slightly bitter taste.

- The abundant starch granules, some simple and spherical but mostly compound with two or more components.
- Crystalline mass of fat was found.
- Inner or ruminating perisperm with oil cells were found with a fragment of vascular tissue.
- the abundant reddish-brown layers of perisperm that form the outer layer and is composed of polygonal cells



Supplementary Fig. S1e. Microscopic images of *Myristica fragrans* spice powder Inner or ruminating perisperm with parts of oil cells and fragment of vascular tissue(a), Layers of Perisperm (b), Inner or ruminating perisperm with oil cells (c), Crystlline masses of fat (d), Crystlline masses of fat (e), Starch granules (f).

Note: All the above characters for all the five spices studied are unique to those plants characteristics will not vary. This helps in finding out the adulteration if any.

Reference

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4. Wijayasiriwardena, C., & Premakumara, S. (2012). Comparative powder microscopy of *Alpinia calcarata* Roscoe and *Alpinia galanga* (Linn.) Willd. *Ayu*, 33(3), 441-442