Tsaoko Fructus

Figure 1 A photograph of Tsaoko Fructus

A. Tsaoko Fructus B. Transverse section C. Groups of seeds D. Seeds

1. NAMES

Official Name: Tsaoko Fructus

Chinese Name: 草果

Chinese Phonetic Name: Caoguo

2. SOURCE

Tsaoko Fructus is the dried ripe fruit of *Amomum tsao-ko* Crevost et Lemarie (Zingiberaceae). The ripe fruit is collected in autumn, foreign matter removed, then dried under the sun to obtain Tsaoko Fructus.

3. DESCRIPTION

Narrowly ellipsoid, obtusely 3-ridged, 1.4-5 cm long, 10-24 mm in diameter. Externally greyish-brown to reddish-brown, with longitudinal grooves and ridges. A rounded and protuberant stylopodium at the apex, and a fruit stalk or its scar at the base. Pericarp tough, easily stripped off longitudinally. Upon removal of the pericarp, 3 groups of seeds visible and separated by yellowish-brown septa in the middle, each group contains 8-20 seeds. Seeds conical to polyhedral, about 5 mm in diameter, externally reddish-brown, covered with greyish-white to yellowish-white membranous aril, a longitudinal furrowed raphe and a dented hilum are located at the apex; texture hard; endosperm greyish-white to yellowish-white. Odour characteristically aromatic; taste pungent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp: Exocarp consists of 1 layer of rectangular cells. Mesocarp broad, consisting of several layers of parenchymatous cells, some cells contain prisms of calcium oxalate. Vascular bundles scattered in the mesocarp, with fibre bundles on the outer side. Endocarp consists of 1 layer of tangentially elongated parenchymatous cells [Fig. 2 (i)].

Seed: Aril consists of several layers of irregularly shaped parenchymatous cells. Epidermal cells of testa rectangular, with relatively thick walls. Hypodermis consists of 1 layer of flat parenchymatous cells, tangentially elongated. Oil cell layer consists of 1 layer of subsquare or rectangular oil cells. Pigment layer consists of several layers of densely packed cells with indistinct boundaries, containing pigments. Endotesta consists of 1 layer of palisade sclerenchymatous cells, with heavily thickened inner and lateral walls, lumen small, containing silica bodies. Perisperm cells palisade-like on the outer layers, filled with small starch granules, some cells contain prisms of calcium oxalate. Endosperm cells contains aleurone grains, surrounding the embryo in the central portion [Fig. 2 (ii)].

Powder

Colour greyish-white to brownish-white. Epidermal cells of testa bright yellow, strip-shaped in surface view, wall thickened, covered with cuticle; bright yellow under the polarized microscope. Endotesta cells yellow to orange-yellow, polygonal or subrounded in surface view, containing silica bodies, wall thickened; cells palisaded in lateral view, with lumen on one side containing silica bodies inside; yellow to orange-yellow under the polarized microscope. Prisms of calcium oxalate 1-16 µm in diameter; bright white or polychromatic under the polarized microscope. Oil cells subrounded, 11-71 µm in diameter. Perisperm cells narrowly rectangular or polygonal, filled with tiny starch granules, some containing prisms of calcium oxalate; pale yellowish-white under the polarized microscope. Exocarp cells rectangular or polygonal in surface view, containing brownish-red pigment. Fibres 9-56 µm in diameter; yellowish-white under the polarized microscope. Vessels mainly scalariform, 5-58 µm in diameter (Fig. 3).

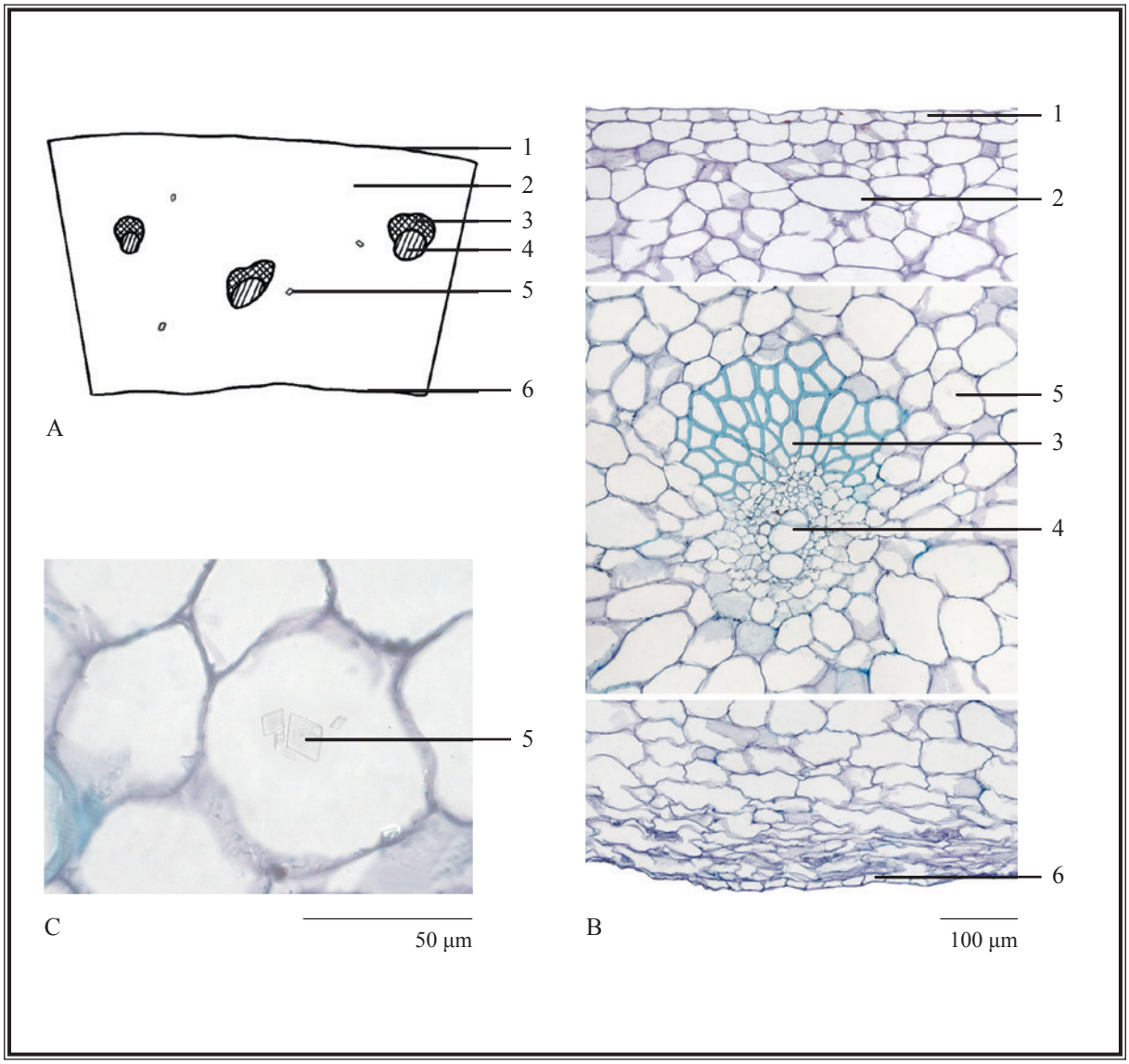

Figure 2 (i) Microscopic features of transverse section of pericarp of Tsaoko Fructus

A. Sketch B. Section illustration C. Prisms of calcium oxalate in parenchyma

1. Exocarp 2. Mesocarp 3. Fibre bundle 4. Vascular bundle
5. Prism of calcium oxalate 6. Endocarp

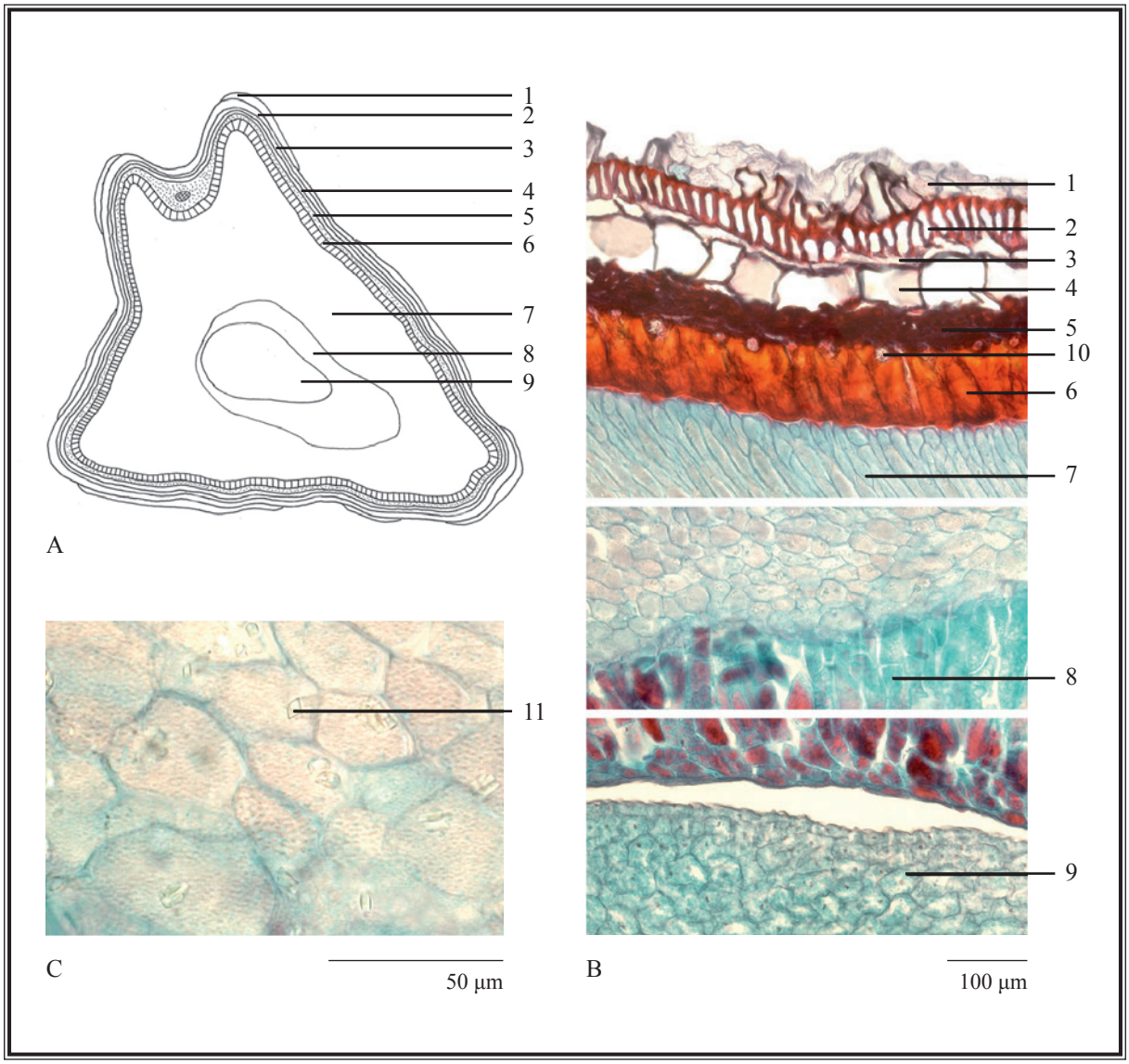

Figure 2 (ii) Microscopic features of transverse section of seed of Tsaoko Fructus

A. Sketch B. Section illustration C. Prisms of calcium oxalate in perisperm

1. Aril 2. Epidermis of testa 3. Hypodermis 4. Oil cell layer
5. Pigment layer 6. Endotesta 7. Perisperm 8. Endosperm
9. Embryo 10. Silica body 11. Prism of calcium oxalate

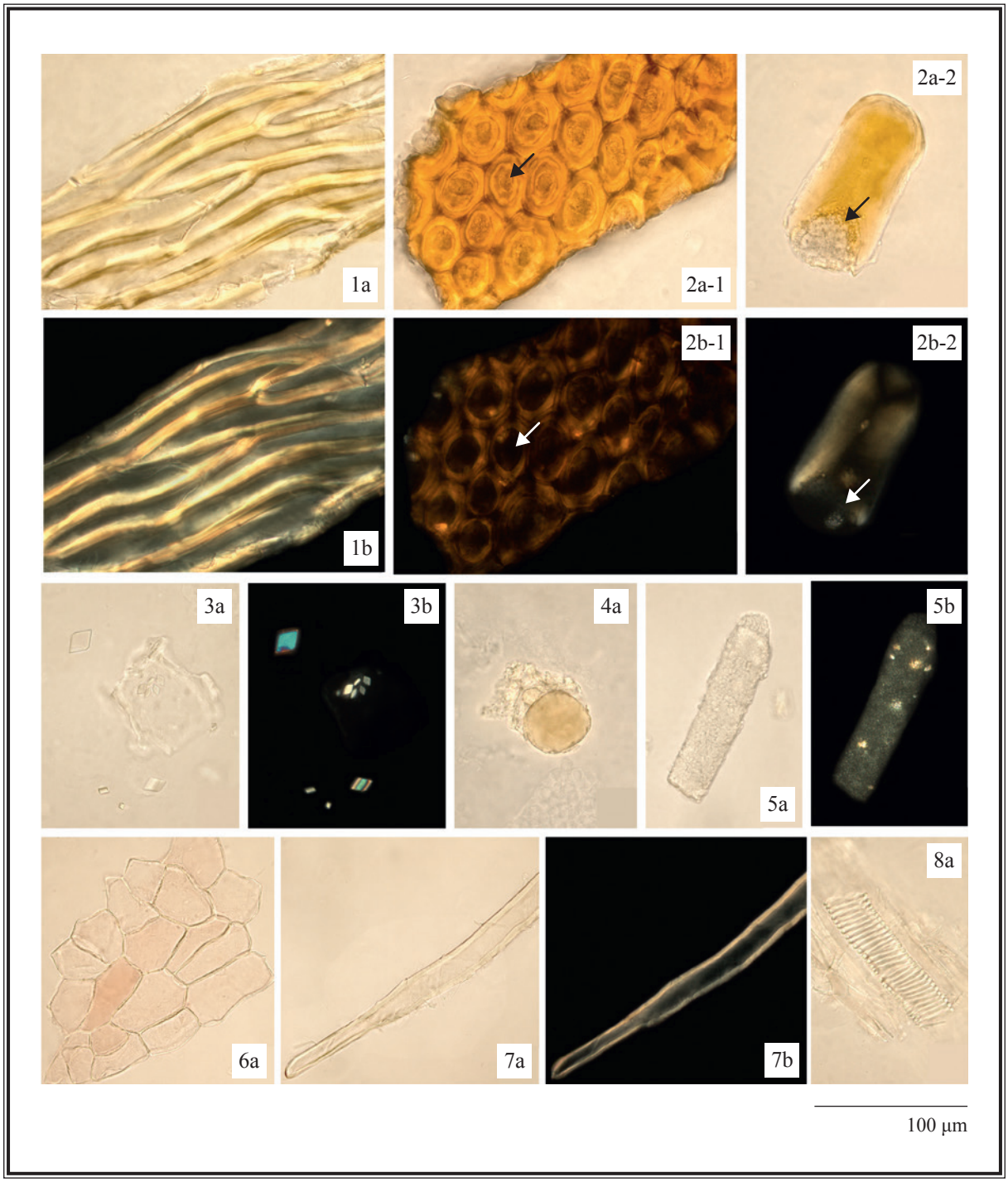

Figure 3 Microscopic features of powder of Tsaoko Fructus

1. Epidermal cells of testa 2. Endotesta cells with silica bodies (2-1 in surface view, 2-2 in lateral view)
3. Prisms of calcium oxalate 4. Oil cell 5. Perisperm cell 6. Exocarp cells 7. Fibre 8. Scalariform vessel

a. Features under the light microscope b. Features under the polarized microscope

4.2 Thin-Layer Chromatographic Identification *[Appendix IV(A)]*

Standard solution

1,8-Cineole standard solution

Weigh 2.0 mg of 1,8-cineole CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of n-hexane, acetone and ethyl acetate (30:1:0.5, v/v).

Spray reagent

Add slowly 1 mL of sulphuric acid to 50 mL of glacial acetic acid and then add 0.5 mL of 4-methoxybenzaldehyde. Freshly prepare the reagent.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2800 × *g* for 10 min. Filter the supernatant.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately 1,8-cineole standard solution (2 µL) and the test solution (8 µL) to the plate. Develop over a path of about 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of 1,8-cineole.

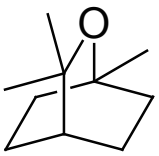

Figure 4 Chemical structure of 1,8-cineole

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

1,8-Cineole standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of 1,8-cineole CRS and dissolve in 10 mL of ethyl acetate.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethyl acetate. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethyl acetate. Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and make up to the mark with ethyl acetate. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-17, 0.25 mm × 30 m) of which the internal wall is covered with (50%-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 250ºC. The detector temperature is at 300ºC. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 5	50	-
5 – 19	50 → 120	5
19 – 31.5	120 → 170	4
31.5 – 39.5	170 → 250	10
39.5 – 41.5	250	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of 1,8-cineole Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of 1,8-cineole should not be more than 5.0%; the RSD of the retention time of 1,8-cineole peak should not be more than 2.0%; the column efficiency determined from 1,8-cineole peak should not be less than 90000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject 1,8-cineole Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention time of 1,8-cineole peak in the chromatogram of 1,8-cineole Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify 1,8-cineole peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 1,8-cineole Std-FP. The retention times of 1,8-cineole peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the five characteristic peaks of Tsaoko Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Tsaoko Fructus extract

Peak No.	RRT	Acceptable Range
1 (α-phellandrene)	0.88	± 0.03
2 (marker, 1,8-cineole)	1.00	-
3 (3,7-dimethyl-6-octenal)	1.59	± 0.03
4 (3,7-dimethyl-2,6-octadienal)	1.69	± 0.03
5 (4-propylbenzaldehyde)	1.86	± 0.03

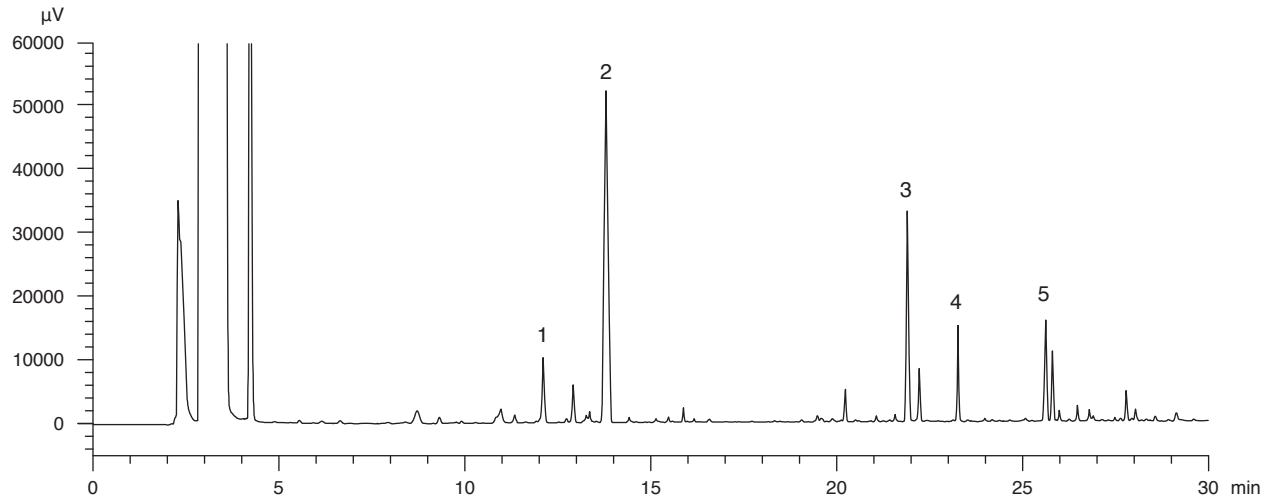

Figure 5 A reference GC fingerprint chromatogram of Tsaoko Fructus extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference GC fingerprint chromatogram (Fig. 5).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 8.0%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 12.0%.
Ethanol-soluble extractives (hot extraction method): not less than 10.0%.

7. ASSAY

7.1 Assay of 1,8-Cineole

Carry out the method as directed in Appendix IV(C).

Standard solution

1,8-Cineole standard stock solution, Std-Stock (1250 mg/L)

Weigh accurately 12.5 mg of 1,8-cineole CRS and dissolve in 10 mL of ethyl acetate.

1,8-Cineole standard solution for assay, Std-AS

Measure accurately the volume of the 1,8-cineole Std-Stock, dilute with ethyl acetate to produce a series of solutions of 10, 20, 50, 100, 200 mg/L for 1,8-cineole.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethyl acetate. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethyl acetate. Centrifuge at about 1800 × *g* for 10 min. Combine the supernatants and make up to the mark with ethyl acetate. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-17, 0.25 mm × 30 m) of which the internal wall is covered with (50%-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 250ºC. The detector temperature is at 300ºC. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0 – 5	50	-
5 – 19	50 → 120	5
19 – 31.5	120 → 170	4
31.5 – 39.5	170 → 250	10
39.5 – 41.5	250	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of 1,8-cineole Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 1,8-cineole should not be more than 5.0%; the RSD of the retention time of 1,8-cineole peak should not be more than 2.0%; the column efficiency determined from 1,8-cineole peak should not be less than 90000 theoretical plates.

The *R* value between 1,8-cineole peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of 1,8-cineole Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of 1,8-cineole against the corresponding concentrations of 1,8-cineole Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify 1,8-cineole peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 1,8-cineole Std-AS. The retention times of 1,8-cineole peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of 1,8-cineole in the test solution, and calculate the percentage content of 1,8-cineole in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.23% of 1,8-cineole ($C_{10}H_{18}O$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 35 g of the powdered seeds by removing the husk and place in a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The seeds contain not less than 1.4% (v/w) of volatile oil.