

ฝาง, แก่น (FANG, KAEN)

ฝางเสน, แก่น (FANG SEN, KAEN), ฝางส้ม, แก่น (FANG SOM, KAEN)

Biancaee Sappan Lignum

Sappan Wood

Synonyms Indian Redwood, Sappanwood

Category Antidiarrheal, anti-inflammatory, hemodynamic.

Sappan Wood is the dried heartwood of *Biancaea sappan* (L.) Tod. (*Caesalpinia sappan* L.) (Family Leguminosae), Herbarium Specimen Number: DMSC 5286, Crude Drug Number: DMSc 1215.

Constituents Sappan Wood contains homoisoflavonoids (e.g., brazilein, brazilin, haematoxylin). It also contains flavonoids, sterols, etc.

Description of the plant (Fig. 1) Tree or scrambling shrub up to 13 m tall; stem and branch with recurved prickles, conspicuous lenticels. Leaves bipinnately compound, alternate, 20 to 45 cm long; petiole 2.5 to 4 cm long; rachis 15 to 40 cm long, often prickly; pinnae 5 to 18 pairs, opposite, 8 to 12 cm long, leaflets 6 to 20 pairs, opposite, oblong or asymmetrical rhombic, 1 to 2.3 cm long, 0.6 to 1 cm wide, apex round to emarginate, base oblique, margin entire, blade papery, both surfaces glabrous or sparsely hairy; petiolule sessile; stipule 3 to 4 mm long, caducous. Inflorescence paniculate or racemose, axillary or terminal, 10 to 40 cm long; bract lanceolate, 5 to 8 mm long, apex acuminate, hairy, caducous. Flower yellow; pedicel 1.5 to 2 cm long, puberulent or pubescent, jointed near top; sepals 5, imbricate, hood-shaped, 0.7 to 1.2 cm long, 3 to 5 mm wide, unequal, lowest one larger than others, glabrous with ciliate margin; petals 5, broadly ovate to obovate, 0.9 to 1.4 cm long, 0.6 to 1 cm wide, unequal, uppermost one clawed, tinged pink at base, hairy inside towards middle; stamens 10, free, slightly exerted, filament densely pubescent at base; ovary superior, elliptic, pubescent, sessile or shortly stalked, 1-loculed, style slender, hairy, stigma truncate; receptacle shallowly campanulate. Fruit a pod, obliquely oblong, widest towards top, 5 to 12 cm long, 1.5 to 4 cm wide, woody, flattened, apex truncate with prominently beaked, base obtuse, reddish brown to brown when aged, shiny. Seeds 2 to 4, light brown, elliptic-oblong, flattened, 1.5 to 1.8 cm long, 0.8 to 1 cm wide.

Description Odour, mild; taste, slightly bitter.

Macroscopical (Fig. 1) Longitudinal pieces of heartwood, orange-yellowish red to reddish, varied in shape and size; surface, rough.

Microscopical (Figs. 2a–2d) Transverse section of the heartwood shows vessels, axial parenchyma, ray parenchyma, fibres, and pith. Vessel: large, thick-walled cells, scattered, solitary or small cluster of 2 to 3 cells, some containing brown substances. Axial parenchyma: polygonal, thick-walled, arranged circularly around the vessel. Ray parenchyma: 1 to 3 rows, some containing brown substances. Fibre: thick-walled. Pith: parenchyma and sclereids; parenchyma, large, elongated, thin-walled, some containing prismatic crystals and/or brown substances; sclereid, large, elongated, thick- and pitted-walled.

Radial longitudinal section of the heartwood shows vessels, axial parenchyma, ray parenchyma, and fibres. Vessel: large, with bordered-pitted and simple perforation plate, some containing brown substances. Axial parenchyma: elongated, thick-walled cells, some containing prismatic crystals, and/or starch grains, and/or brown substances. Ray parenchyma: multiseriate, thick-walled, rectangular cells, some containing prismatic crystals, and/or brown substances, and/or starch grains. Fibre: thick-walled.

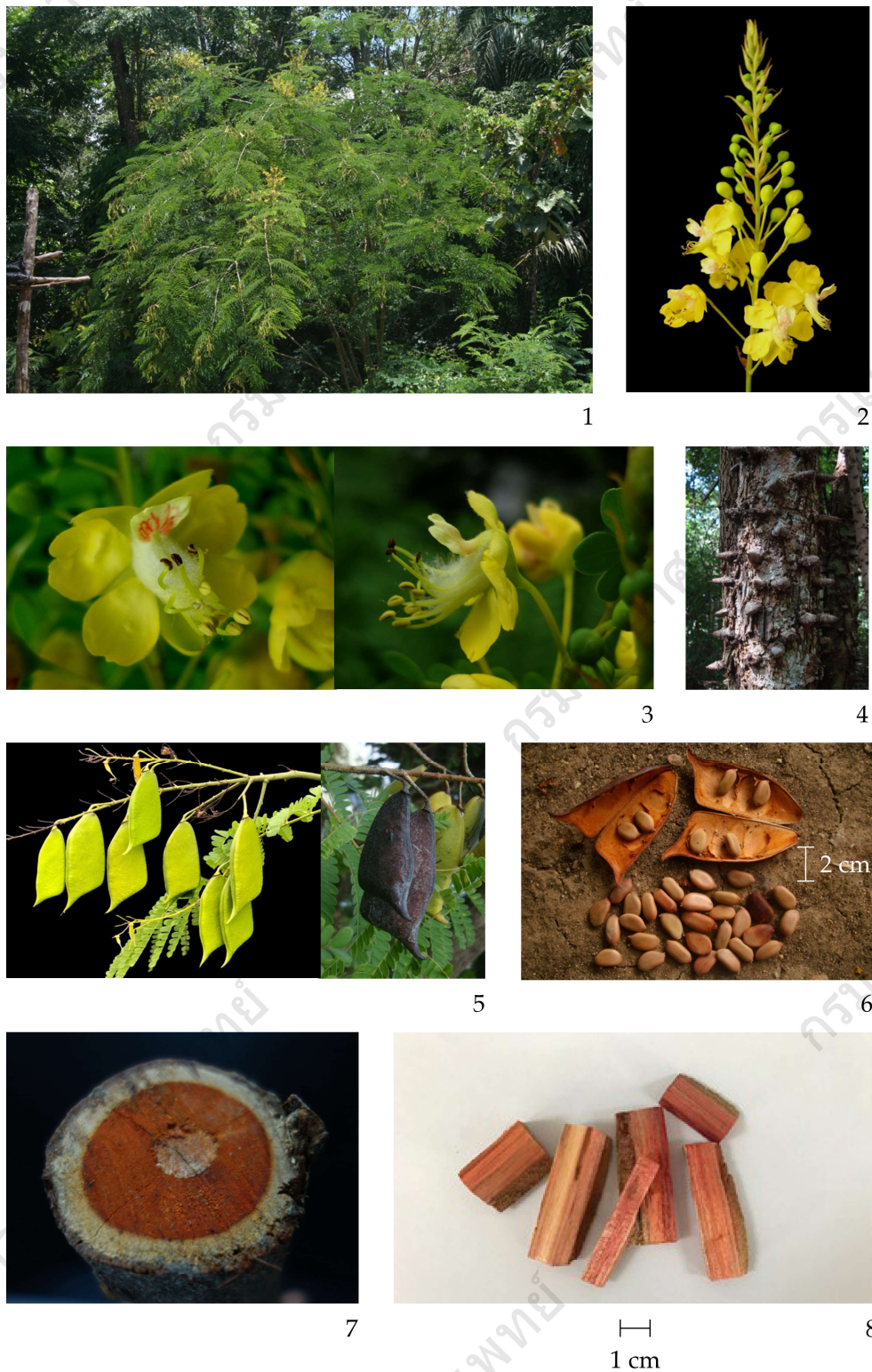


Fig. 1 *Biancaea sappan* (L.) Tod.

1. habit 2. inflorescence 3. flowers 4. stem showing prickles 5. fruits
 6. seeds 7. cross section of stem showing bark, sapwood, and heartwood 8. crude drug

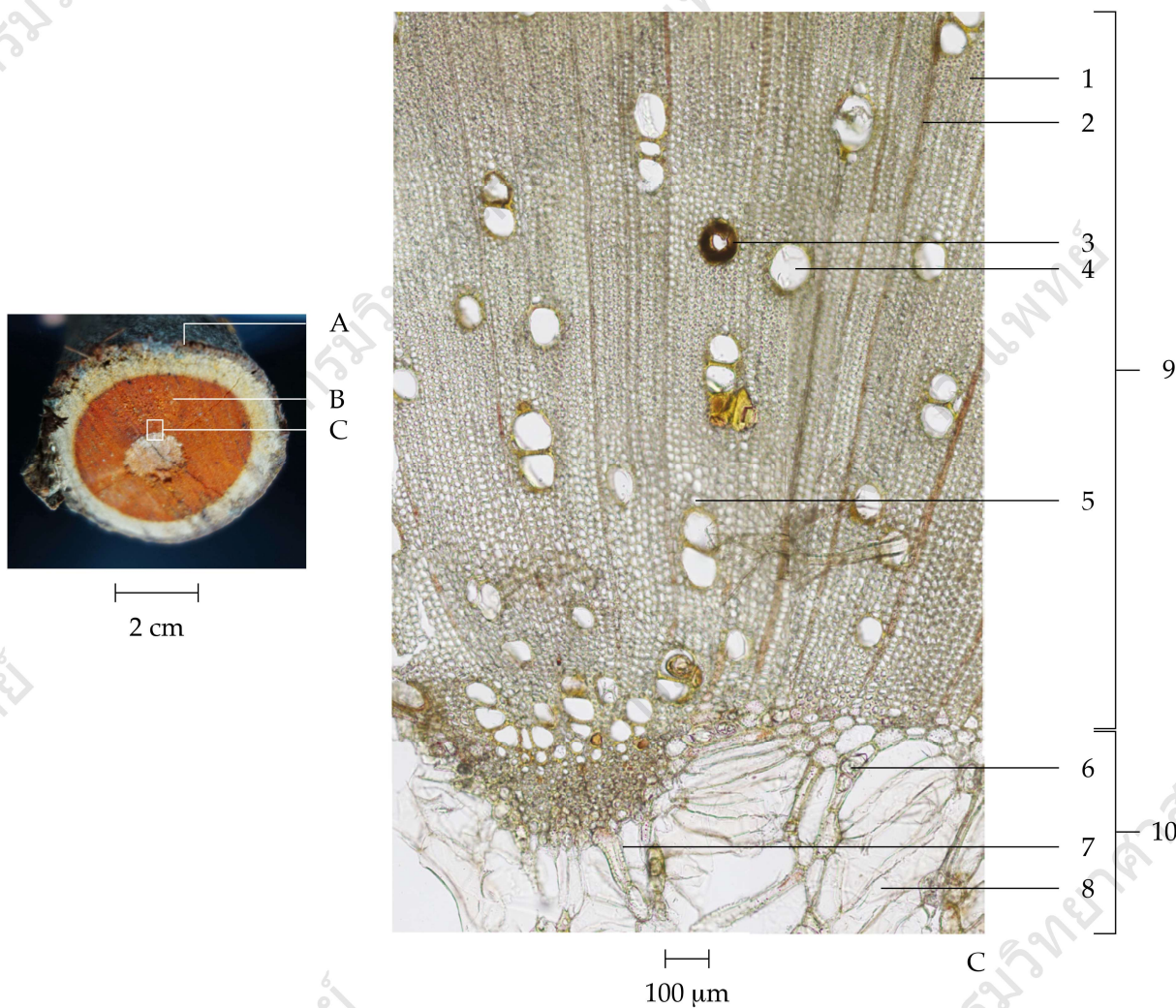


Fig. 2a Photomicrograph of Transverse Section of the Heartwood of *Biancaea sappan* (L.) Tod.

- A. Bark
B. Heartwood
C. Heartwood and Pith

- | | |
|---------------------|----------------------|
| 1. fibre | 6. prismatic crystal |
| 2. ray parenchyma | 7. sclereid |
| 3. brown substance | 8. parenchyma |
| 4. vessel | 9. heartwood |
| 5. axial parenchyma | 10. pith |

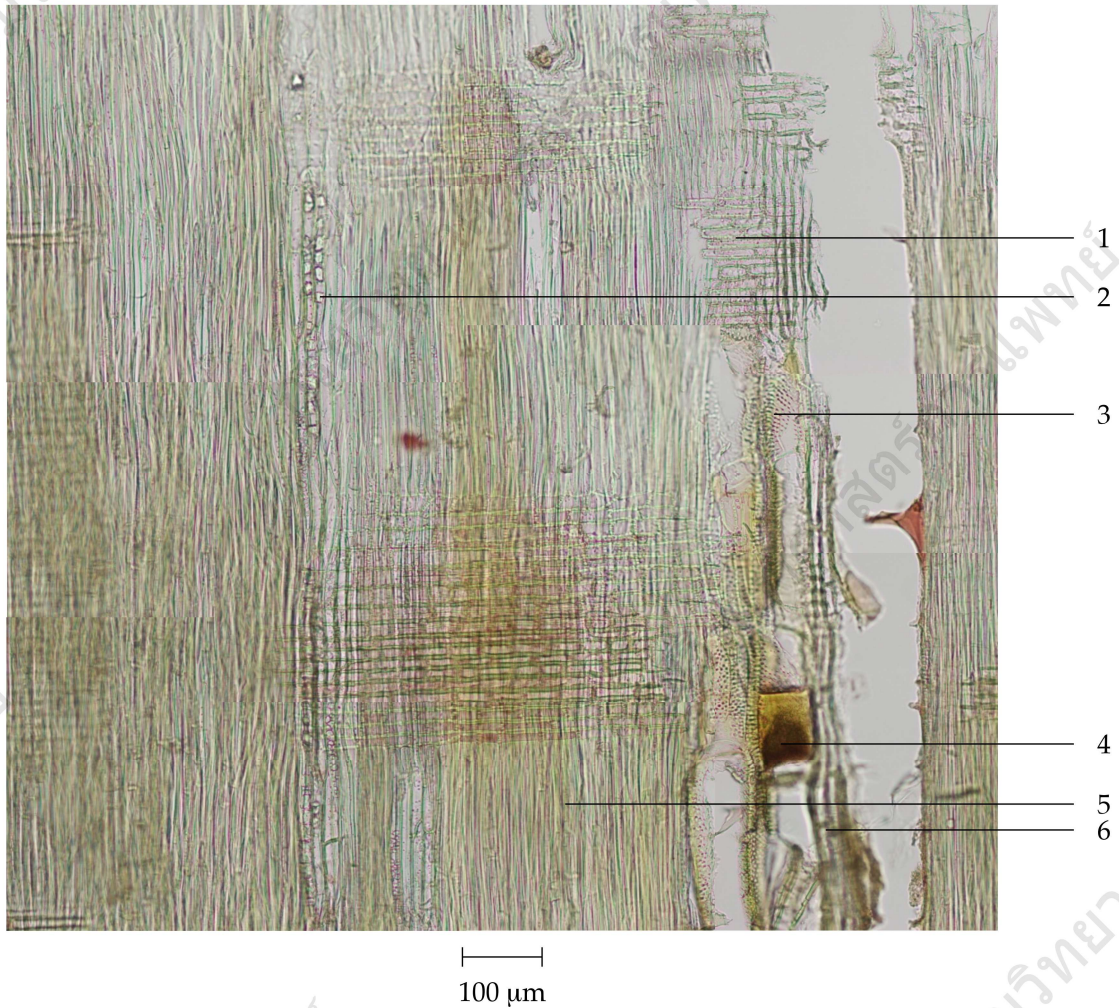


Fig. 2b Photomicrograph of Radial Longitudinal Section of the Heartwood of *Biancaea sappan* (L.) Tod.

- | | |
|---------------------------|---------------------|
| 1. ray parenchyma | 4. brown substance |
| 2. prismatic crystal | 5. fibre |
| 3. bordered-pitted vessel | 6. axial parenchyma |

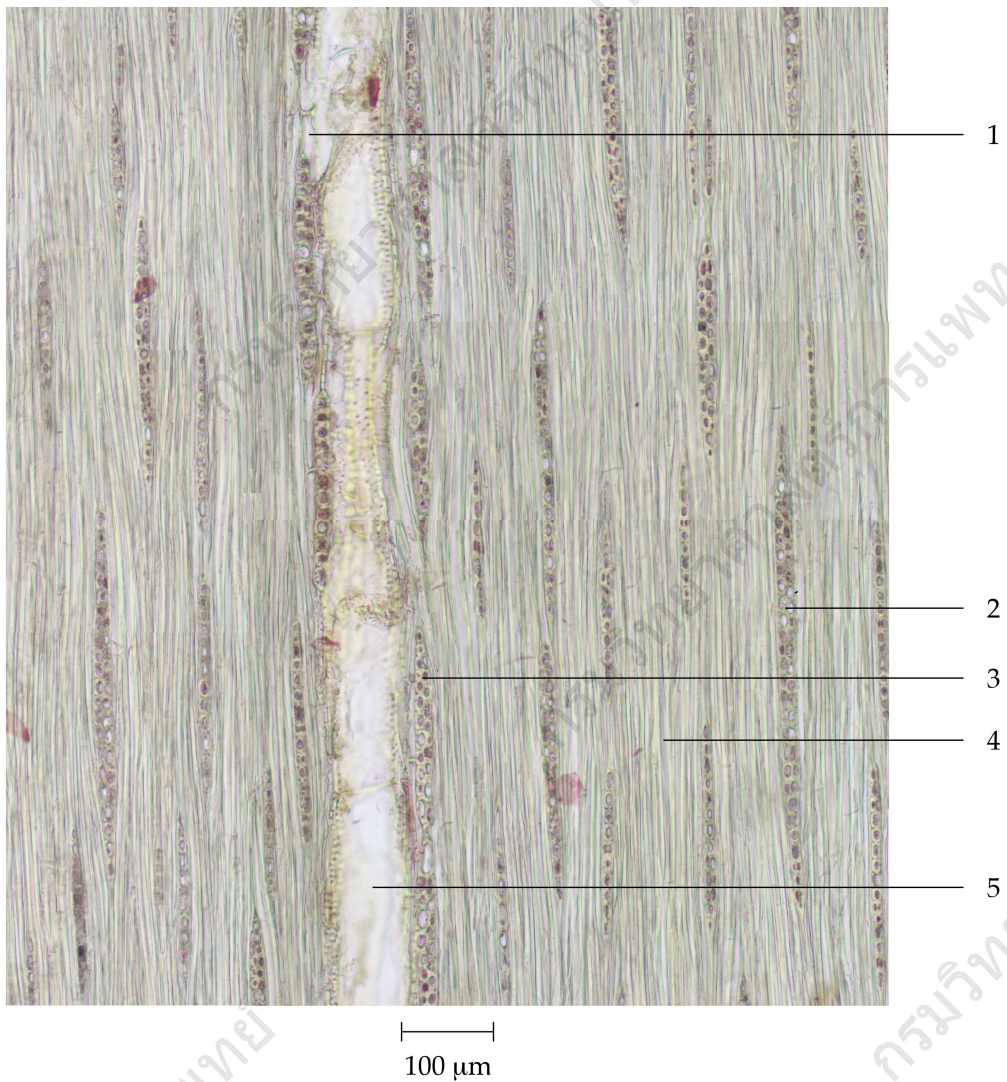


Fig. 2c Photomicrograph of Tangential Longitudinal Section of the Heartwood of *Biancaea sappan* (L.) Tod.

- | | |
|---------------------|-----------|
| 1. axial parenchyma | 4. fibre |
| 2. ray parenchyma | 5. vessel |
| 3. brown substance | |

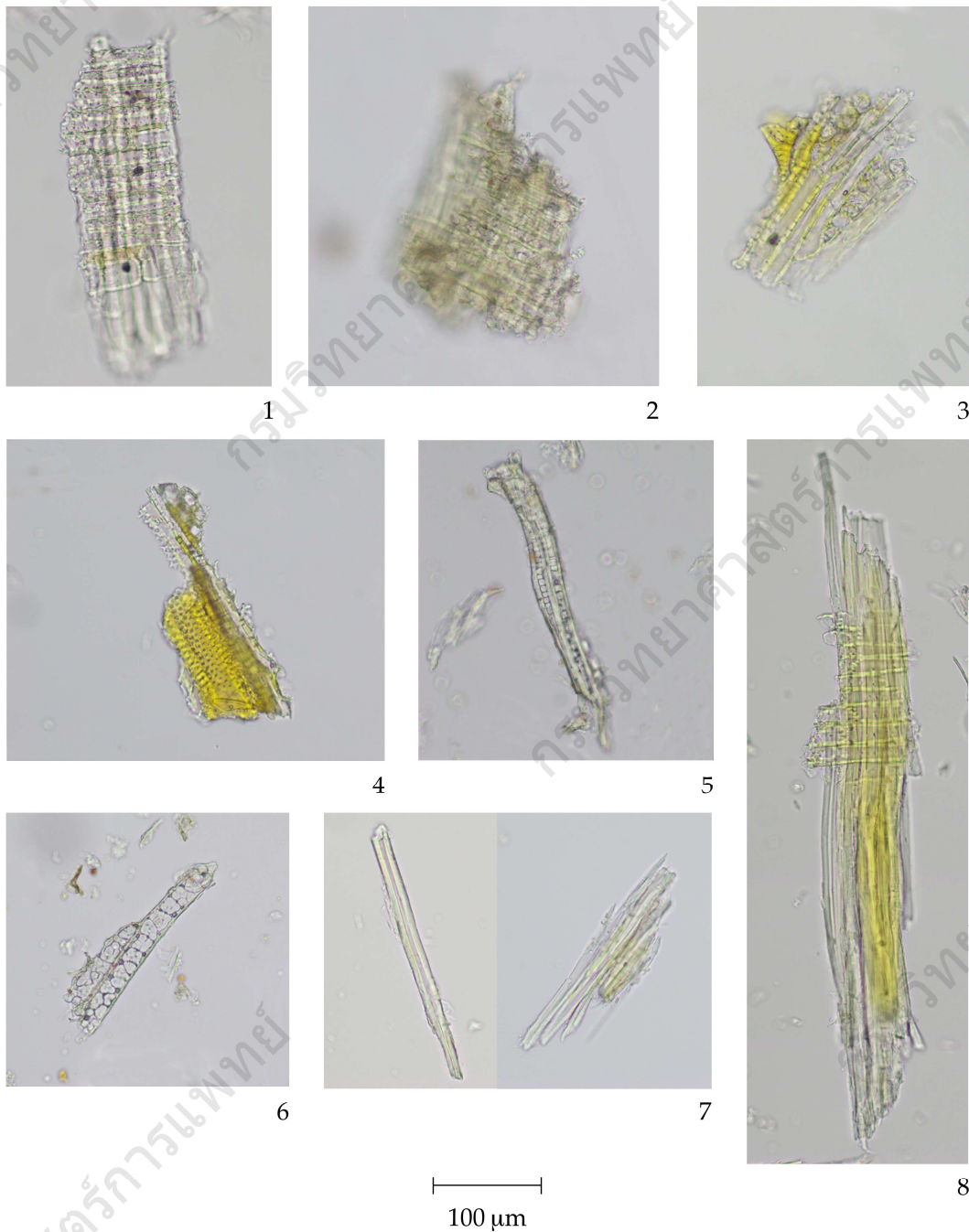


Fig. 2d Photomicrographs of Powdered Drug of the Heartwood of *Biancaea sappan* (L.) Tod.

1. ray parenchyma and underlying fibres, in radial longitudinal view
2. ray parenchyma, some containing starch grains
3. fibres associated with ray parenchyma, containing starch grains, in tangential longitudinal view
4. bordered-pitted vessels associated with axial parenchyma and fibres
5. fragment of fibres containing starch grains and prismatic crystals
6. parenchyma, in longitudinal view, containing starch grains
7. fibres
8. ray parenchyma adjacent to fibres, in radial longitudinal view

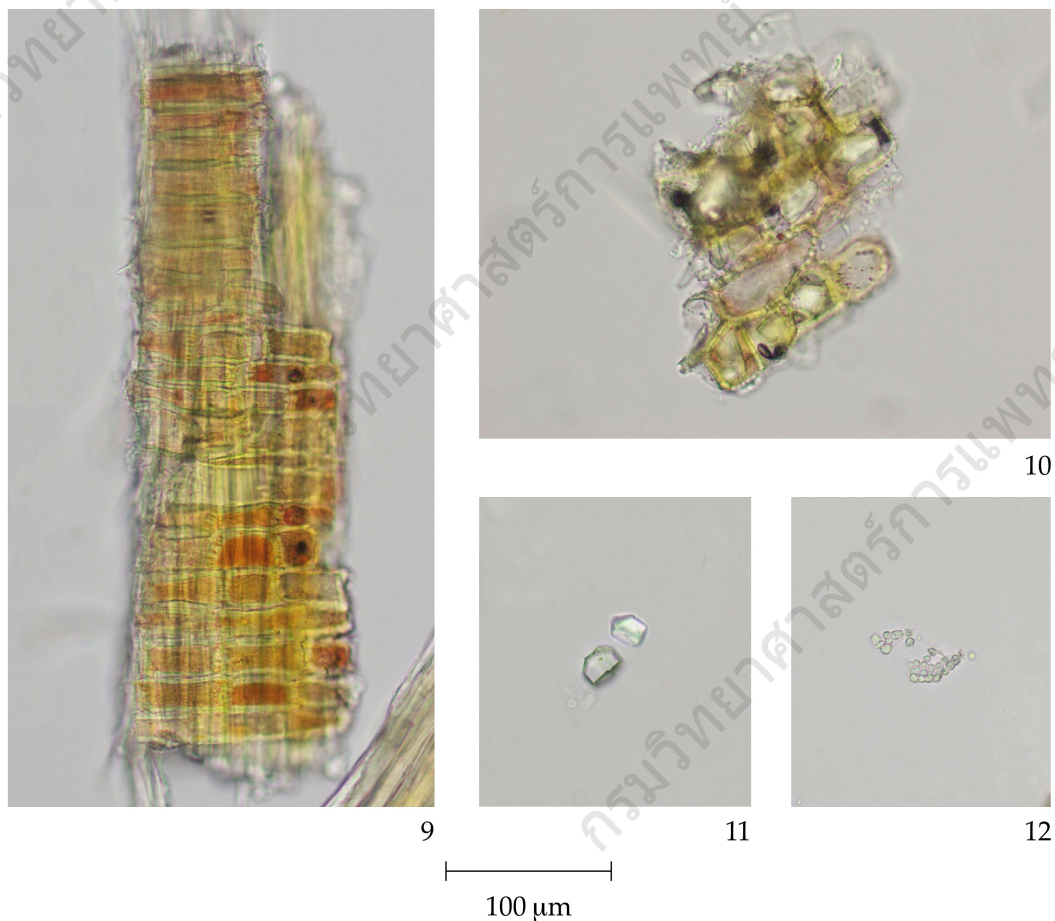


Fig. 2d (continued)

- 9. ray parenchyma, some containing red to brown substances, and underlying fibres, in radial longitudinal view
- 10. sclereids, some containing prismatic crystals, starch grains, and red to brown substances

- 11. prismatic crystals
- 12. starch grains

Tangential longitudinal section of the heartwood shows vessels, axial parenchyma, ray parenchyma, and fibres. Vessel: large, with bordered-pitted and simple perforation plate, some containing brown substances. Axial parenchyma: thick-walled, elongated cells, some containing prismatic crystals, and/or starch grains, and/or brown substances. Ray parenchyma: 1 to 3 rows, thick-walled, oval cells, some containing brown substances. Fibre: thick-walled.

Sappan Wood in powder possesses the diagnostic microscopical characters of the unground drug. Fibres containing starch grains and ray parenchyma containing red to brown substances are characteristics. Various views of ray parenchyma and large bordered-pitted vessels are commonly observed. Starch grains, prismatic crystals, and red to brown substances can also be seen.

Packaging and storage Sappan Wood shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Macerate 500 mg of the sample, in *fine powder*, with 10 mL of *ethanol* for 5 minutes and filter. To 2 mL of the filtrate, add 2 mL of a 1 per cent w/v solution of *sodium carbonate* and mix: a dark red colour develops.

B. Macerate 100 mg of the sample, in *fine powder*, with 10 mL of *ethanol* for 15 minutes and filter. Mix 2 mL of the filtrate with 2 or 3 pieces of *magnesium ribbon* and a few drops of *hydrochloric acid*: a pink colour develops.

C. The chromatogram of the Sample preparation shows several peaks, one of which corresponds to the brazilin peak of the Standard preparation, as obtained in the *Brazilin* content (Fig. 3).

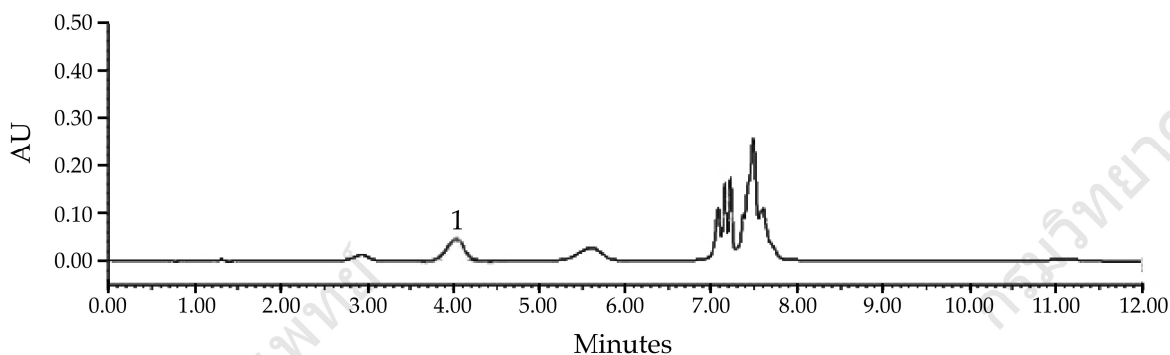


Fig. 3 HPLC Chromatogram of Sappan Wood Showing Brazilin (1)

D. Carry out the test as described in the “Thin-Layer Chromatography” (Appendix 3.1), using a high-performance plate with *silica gel GF254* as the coating substance and a mixture of 60 volumes of *chloroform*, 40 volumes of *acetone*, and 5 volumes of *formic acid* as the mobile phase and allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate as bands of 8 mm, 5 μ L of solution (A) and 3 μ L of solution (B). Prepare solution (A) by macerating 100 mg of the sample, in *fine powder*, with 5 mL of *methanol* for 10 minutes and filtering. For solution (B) dissolve 1 mg of *brazilin* in 5 mL of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 49 to 53) corresponding to brazilin from solution (B) and other three quenching bands are also observed. Heat the plate at 80° for 10 minutes and

then spray with *natural products* (NP) TS while the plate is still warm. Subsequently spray the plate with *polyethyleneglycol* (PEG) TS and observe the colours of the bands under ultraviolet light (366 nm) through the cut-off filter within 5 to 15 minutes; the band due to brazilin is red-brown fluorescent. One red-brown, one yellow, and two dark fluorescent bands are also observed (Fig. 4).

Loss on drying Not more than 7.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 1.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 7.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Brazilin content Not less than 0.7 per cent w/w of brazilin (C₁₆H₁₄O₅). Carry out the determination as described in the “Liquid Chromatography” (Appendix 3.5).

Mobile phase A Use *methanol*.

Mobile phase B Prepare a 0.3 per cent v/v solution of *glacial acetic acid*.

Standard preparation Dissolve an accurately weighed quantity of Brazilin RS in *methanol* to obtain a solution containing 200 µg per mL.

Sample preparation Sonicate about 100 mg of Sappan Wood, in *fine powder* and accurately weighed, with 10.0 mL of *methanol* for 30 minutes and filter through a membrane having a 0.45-µm porosity.

The step gradient of mobile phases is as follows:

Time (Minutes)	Mobile Phase A (Per Cent V/V)	Mobile Phase B (Per Cent V/V)
0	23	77
5	23	77
6	100	0
9	100	0
10	23	77
12	23	77

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (2.7 µm), equipped with a similarly packed guard column and maintained at a temperature of 27°, (b) *Mobile phase* at a flow rate of about 1.4 mL per minute, and (c) an ultraviolet photometer set at 290 nm.

To determine the suitability of the chromatographic system, chromatograph *Standard preparation* and record the peak response as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0 per cent.

Procedure Separately inject about 10 µL each of *Standard preparation* and *Sample preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculation Calculate the content of C₁₆H₁₄O₅ in the portion of the Sappan Wood taken, using the declared content of C₁₆H₁₄O₅ in Brazilin RS.

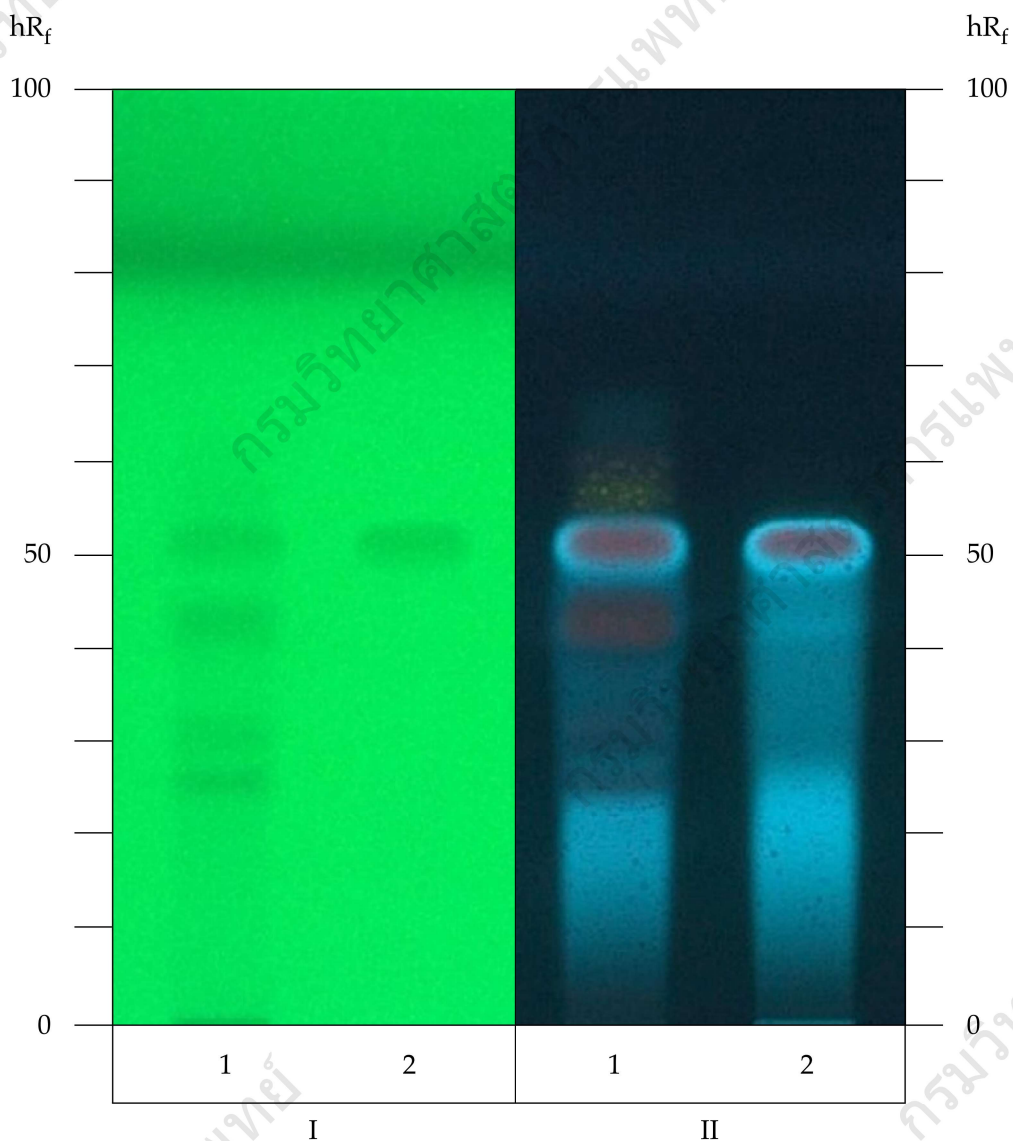


Fig. 4 Thin-Layer Chromatogram of Methanolic Extract of the Heartwood of *Biancaea sappan* (L.) Tod.

1 = solution (A)

2 = solution (B)

I = detection under UV light (254 nm)

II = detection under UV light (366 nm) after spraying with NP/PEG TS