สารสกัดแห้งฝาง (FANG DRY EXTRACT)

Sappan Wood Dry Extract

Category Sappan Wood Antidiarrheal, anti-inflammatory, hemodynamic.

Sappan Wood Dry Extract is prepared from the powdered Sappan Wood by extraction with *ethanol*. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of brazilin ($C_{16}H_{14}O_5$); the labelled amount of brazilin is not less than 3.0 per cent, calculated on the dried basis.

Description Brownish yellow powder.

Packaging and storage Sappan Wood Dry Extract shall be kept in tightly closed containers, protected from light, and stored in a cool and dry place.

Labelling The label on the container states (1) the amount of brazilin; (2) the expiration date.

Identification

A. Dissolve about 10 mg of the sample, in powder, in 10 mL of *ethanol*. To 2 mL add 2 mL of a 1 per cent w/v solution of *sodium carbonate* and mix: a pinkish red colour develops.

B. The chromatogram of the Assay preparation shows several peaks, one of which corresponds to that of the Standard preparation, as obtained in the *Assay* (Fig. 1).

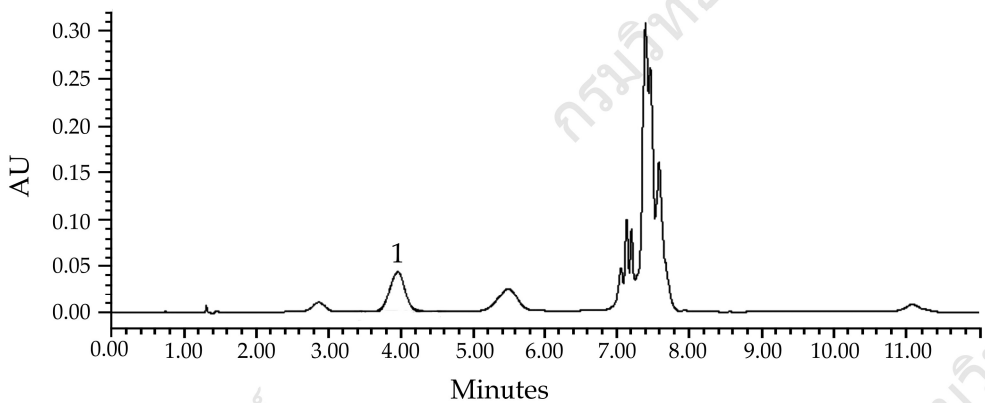

Fig. 1 HPLC Chromatogram of Sappan Wood Dry Extract Showing Brazilin (1)

C. 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 to the plate as bands of 8 mm, 5 µL of solution (A) and 3 µL of solution (B). Prepare solution (A) by dissolving 10 mg of the sample, in powder, in 5 mL of *methanol*. 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 46 to 49) 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 dark, one yellow, and two red-brown fluorescent bands are also observed (Fig. 2).

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Assay 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 preparations Dissolve an accurately weighed quantity of Brazilin RS in sufficient *methanol*, dilute quantitatively and stepwise with *methanol* to obtain a stock solution having a known concentration of about 200 μg per mL. Dilute the solution quantitatively and stepwise with *methanol* to obtain six solutions having known concentrations of 10, 40, 80, 120, 160, and 200 μg per mL.

Assay preparation Dissolve about 10 mg of Sappan Wood Dry Extract, accurately weighed, in 10.0 mL of *methanol*, mix well, and filter through a 0.45-μm membrane filter.

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 (5 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.

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	12	77

To determine the suitability of the chromatographic system, chromatograph *Standard* preparation having a known concentration of 120 μg per mL 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 preparations* into the chromatograph, record the chromatograms, and measure the responses for brazilin peaks. Plot the readings and draw the standard curve of best fit: the curve shows a correlation coefficient of not less than 0.999. Inject about 10 μL of *Assay preparation* into the chromatograph, record the chromatogram, and measure the response for the major peak.

Calculation By reference to the standard curve, calculate the content of brazilin ($C_{16}H_{14}O_5$) in the portion of the Extract taken.

Other requirements Complies with the requirements described under "Extracts" (Appendix 1.16H).

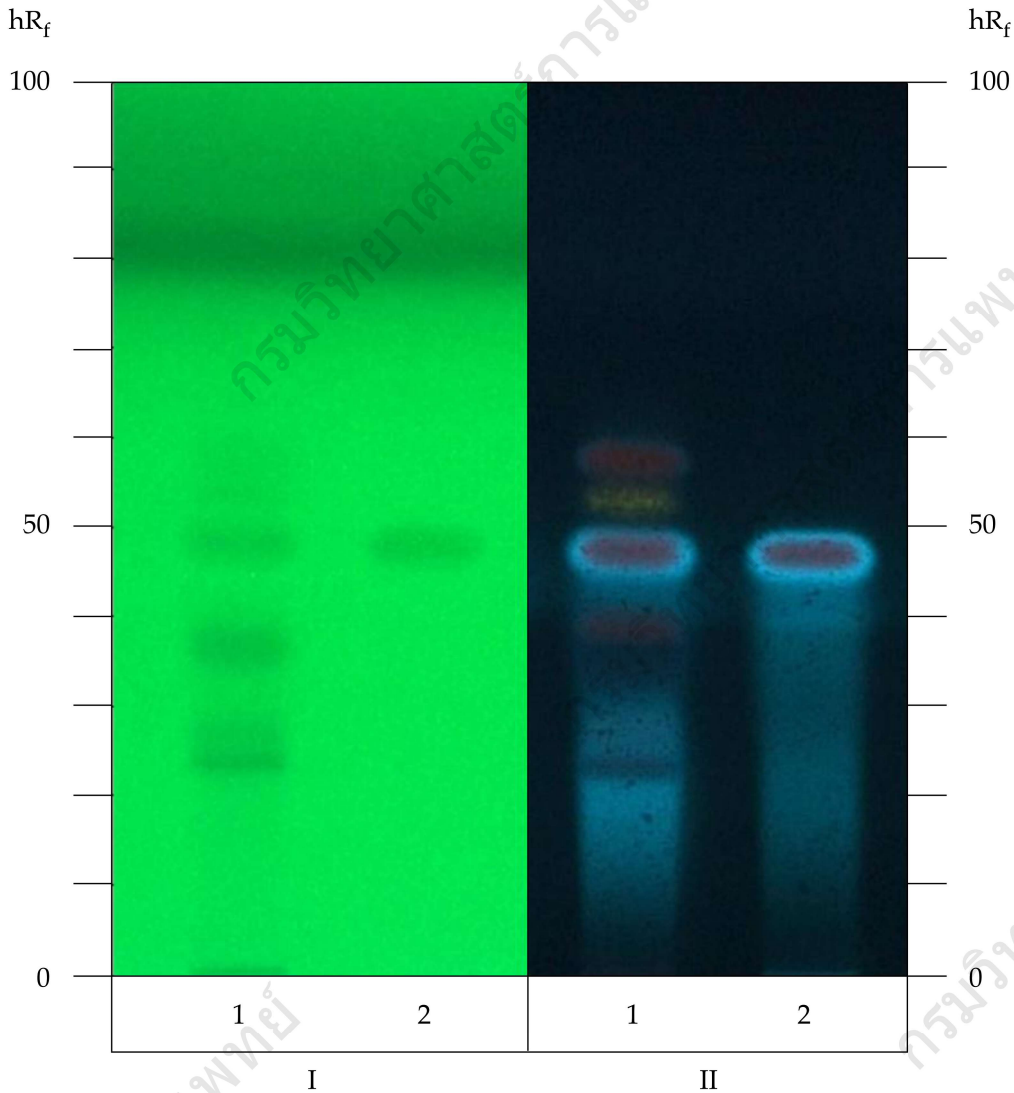

Fig. 2 Thin-Layer Chromatogram of Sappan Wood Dry Extract
1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm) after spraying with NP/PEG TS