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Maritime Pine

DEFINITION

Maritime Pine consists of the bark of stems of *Pinus pinaster* Aiton (*Pinus maritima* Poir.) Fam. Pinaceae. It contains NLT 8.0% and NMT 12.0% of procyanidins, calculated on the dried basis.

[NOTE—This article is intended to be used in the preparation of extracts only and is not for direct human consumption.]

IDENTIFICATION

• A. PRESENCE OF PROCYANIDINS

Sample: Pulverize 1 g of the dried Maritime Pine. Use 10 mg.

Analysis: Add the *Sample* to 1 mL of methanol, and add 6 mL of a mixture of butanol and hydrochloric acid (95:5). Heat for 2 min in a water bath.

Acceptance criteria: The solution turns red.

• B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution: 25 mg/mL of [USP Maritime Pine Extract RS](#) in alcohol

[NOTE—Retain a portion of this solution for use in *Identification* test C.]

Sample solution: Add 2 g of the powdered dried material to 20 mL of water. Place in a water bath for 20 min, and centrifuge. Extract the supernatant with 40 mL of ethyl acetate. Evaporate the ethyl acetate layer to dryness under a stream of nitrogen, with gentle heating. Dissolve the residue so obtained in 0.25 mL of alcohol.

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Ethyl acetate, formic acid, and water (50:5:3)

Spray reagent: Alcohol and phosphoric acid (1:1), containing 1% of vanillin

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatograms, dry the plate with the aid of a current of air, spray the plate with the *Spray reagent*, and heat at 115° for 15 min.

Acceptance criteria: The chromatogram of the *Sample solution* presents three red bands appearing in the middle third of the chromatogram corresponding to two dimeric procyanidins and catechin at the same R_f of similar bands in the *Standard solution*. The chromatogram of the *Sample solution* also exhibits a blue band between the upper band due to upper dimeric procyanidins and the band due to catechin, at the same R_f of a similar band found in the chromatogram of the *Standard solution*.

• C. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution A: Use the *Standard solution*, prepared as directed for *Identification* test B.

Standard solution B: 1 mg/mL each of ferulic acid and protocathechuic acid

Sample solution: Use the *Sample solution*, prepared as directed for *Identification* test B.

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Methylene chloride, methanol, glacial acetic acid, and water (80:15:2:2)

Spray reagent: 5% ferric chloride solution in methanol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram, dry the plate at 110°, and examine the plate under short-wavelength and long-wavelength UV light. The chromatograms of *Standard solution A* and *Standard solution B* exhibit bands in the middle third and upper third that correspond to

protocatechuic acid and ferulic acid, respectively. Spray the plate with the *Spray reagent*, and heat at 115° for 15 min. The bands due to ferulic acid and protocatechuic acid turn grayish green. Grayish-green bands become visible in the chromatogram of *Standard solution A* above and below protocatechuic acid, indicating the presence of caffeic acid and catechin, respectively.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits bands due to catechin, protocatechuic acid, caffeic acid, and ferulic acid that correspond in color and R_f values to those in the chromatogram of *Standard solution A* and *Standard solution B*.

COMPOSITION

• CONTENT OF PROCYANIDINS

Reagent solution A: Butanol and hydrochloric acid (95:5)

[NOTE—Prepare this solution on the day of use.]

Reagent solution B: Dissolve 2 g of ferric ammonium sulfate in a mixture of 100 mL of water and 17.5 mL of hydrochloric acid. [NOTE—This solution can be used within 15 days of preparation.]

Standard solution: 95 µg/mL of procyanidins from [USP Maritime Pine Extract RS](#) in methanol

Sample stock solution: Dry crushed Maritime Pine at 110° for 3 h. Place 1.9 g of the crushed material in a 20-mL vial, and add 10 mL of methanol. Crimp the vial, and sonicate for 2 min. Heat in boiling water for 10 min. Cool to room temperature, allow the sediment to settle, and transfer the supernatant to a 100-mL volumetric flask, passing it through a filter having a 0.45-µm pore size. Wash the sediment two times with 10 mL of methanol, and transfer the solution into the same 100-mL volumetric flask, again passing it through a filter having a 0.45-µm pore size. Dilute with methanol to volume.

Sample solution: Dilute the *Sample stock solution* with methanol (1 in 20).

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: Vis

Wavelength: 551 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer 1.0 mL each of the *Standard solution*, *Sample solution*, and methanol to three separate 10-mL vials. To each vial add 6.0 mL of *Reagent solution A* and 0.25 mL of *Reagent solution B*. Seal the vials with crimp caps. Mix, and heat in a water bath for 40 min. Quickly cool to room temperature in an ice bath. Quantitatively transfer these solutions, with the aid of *Reagent solution A*, to three separate 10-mL volumetric flasks, and dilute with *Reagent solution A* to volume.

Determine the absorbance of the solutions obtained from the *Standard solution* and the *Sample solution*, using the methanol-containing solution as a blank.

Calculate the percentage of total procyanidins in the portion of Maritime Pine taken:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/W) \times D \times P$$

A_U = absorbance of the solution from the *Sample solution*

A_S = absorbance of the solution from the *Standard solution*

C_S = concentration of the [USP Maritime Pine Extract RS](#) in the *Standard solution* (mg/mL)

V = volume of the *Sample stock solution* (mL)

W = weight of Maritime Pine taken to prepare the *Sample stock solution* (mg)

D = dilution factor to prepare the *Sample solution* from *Sample stock solution*, 20

P = percentage of procyanidins in the [USP Maritime Pine Extract RS](#)

Acceptance criteria: 8.0%–12.0% on the dried basis

SPECIFIC TESTS

• BOTANICAL CHARACTERISTICS

Macroscopic: Bark pieces are typically 1–3 cm thick. The inner bark is plane to slightly concave, whitish to light brown, striped longitudinally; shiny and of slightly irregular surface, only a few millimeters thick. Abrupt change to a sequence of hard, convex, nearly parallel layers alternating with smooth, light brown layers. Up to 50 or more layers present, depending on the age of the bark. Outer surface of bark is dark reddish brown composed of irregular scaly patches, with deep V-shaped fissures. Outer surface may also be gray, gray-green, or green-yellow due to presence of lichens.

Microscopic (transverse section of bark): Light inner bark has irregular lateral stripes consisting of 3–5 cell layers of long, slender sieve cells with large pitted horizontal cell walls and large polygonal parenchyma cells containing single, irregular, rounded starch gran, 3–15 µm