

Ginkgo

Ginkgo consists of the dried leaf of *Ginkgo biloba* Linn é (Fam. Ginkgoaceae). It contains not less than 0.5 percent of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7; and not less than 0.1 percent of terpene lactones, calculated as the sum of bilobalide ($C_{15}H_{18}O_8$), ginkgolide A ($C_{20}H_{24}O_9$), ginkgolide B ($C_{20}H_{24}O_{10}$), and ginkgolide C ($C_{20}H_{24}O_{11}$), both on the dried basis.

Packaging and storage— Preserve in well-closed containers, protected from light and moisture, and store at room temperature.

Labeling— The label states the Latin binomial and, following the official name, the part of the plant contained in the article.

Botanic characteristics—

Macroscopic: Dried whole, folded, or fragmented leaves, with or without attached petiole, varying from khaki green to greenish brown in color, often more brown at the apical edge, and darker on the adaxial surface. Lamina broadly obtuse (fan-shaped), 2 to 12 cm in width and 2 to 9.5 cm in length from petiole to apical margin; mostly 1.5 to 2 times wider than long. Base margins entire, concave; apical margin sinuate, usually truncate or centrally cleft, and rarely multiply cleft. Surface glabrous, with wrinkled appearance due to prominent dichotomous venation appearing parallel and extending from the lamina base to the apical margin. Petiole of a similar color to leaf, channeled on the adaxial surface, 2 to 8 cm in length.

Histology—

Transverse section of lamina: A thin but marked cuticle occurs over a single layer of epidermal cells on both surfaces. Stomata are present on the lower surface only, with guard cells sunken with respect to adjacent epidermal cells. Palisade elements, elongated at right angles to the surface and often irregular in appearance, occur just below the upper epidermis. Vascular bundles occur at intervals along the width of the blade, with adjacent cluster crystals of calcium oxalate. Cells of the mesophyll are smaller than the palisade cells, elongated parallel to the leaf surface and separated by large intercellular spaces.

Powdered lamina and petiole: Under the microscope, transverse fragments of the leaf display a smooth cuticle, present on both leaf surfaces and staining pinkish orange with [sudan III TS](#). In surface view, cells of the upper epidermis are elongated and wavy-walled, with abundant yellow

droplets 2 to 12 μm in diameter visible in mature and old leaves but not in young leaves. Cells of the lower epidermis are similar in shape but have straighter walls and are interrupted by anisocytic stomata. Numerous lignified elements derived from the lamina and petiole are present, including xylem vessels with annular thickening, tracheids, and vessels with bordered pits. The extent of lignification, particularly in the petiole, increases with age of leaf. Calcium oxalate crystals are numerous, present scattered or associated with vessels, ranging in size from 5 to 50 μm in young leaves to 15 to 100 μm in mature leaves. Under crossed polaroids, numerous smaller prism- or tear-shaped shiny features of indeterminate nature may be present. Very occasional, highly elongated, uniseriate, covering trichomes with no obvious cross walls and smooth or warty surfaces may be seen. Mature leaves may show the presence of very rare, polygonal to circular starch granules approximately 20 μm in diameter, with a central hilum and exhibiting a marked Maltese cross under crossed polaroids.

[Thin-layer chromatographic identification test](#) 201

Test for flavonoids—

Test solution— Transfer 0.2 g of finely powdered Ginkgo to a test tube, add 10 mL of methanol, and heat on a water bath at 65 °C for 10 minutes. Shake the mixture frequently during the heating.

Allow to cool to room temperature, filter, concentrate the filtrate on a hot water bath at 60 °C to half its volume, and cool.

Standard solution: a solution of USP Rutin RS and [USP Chlorogenic Acid RS](#) in methanol containing about 0.6 mg per mL and 0.2 mg per mL, respectively.

Developing solvent system: a mixture of ethyl acetate, water, anhydrous formic acid, and glacial acetic acid (67.5:17.5:7.5:7.5).

Spray reagent 1: a solution of 2-aminoethyl diphenylborinate in methanol containing 10 mg per mL.

Spray reagent 2: a solution of polyethylene glycol 400 in alcohol containing 50 mg per mL.

Procedure— Apply separately, as bands, 20 μL each of the Test solution and the Standard solution to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel, and allow the bands to dry. Develop the chromatograms in the Developing solvent system until the solvent front has moved about 10 cm from the origin.

Remove the plate from the chromatographic chamber, and dry it in a circulating air oven at 100 to 105 °C. Immediately spray the hot plate with Spray reagent 1, and then spray with Spray reagent 2. Allow the plate to cool for 30 minutes, and examine it under long-wavelength UV light. The chromatogram of the Standard solution shows in its middle part, with increasing R_F values, the yellowish-brown fluorescent zone due to rutin and a light blue fluorescent zone due to chlorogenic acid. The chromatogram of the Test solution shows a yellowish-brown fluorescent zone and a light blue fluorescent zone at R_F similar to those of rutin and chlorogenic acid, respectively, in the chromatogram of the Standard solution. Additional yellowish-green zones due to flavonoids are detected in the chromatogram of the Test solution. These include one zone below the rutin zone, two zones between the rutin and chlorogenic acid zones, and four zones above the chlorogenic acid zone. Other, less intense zones may be seen in the chromatogram of the Test solution.

Test for terpene lactones—

Adsorbent: a 0.50-mm layer of chromatographic silica gel.

Test solution— Transfer 0.8 g of the dried test specimen retained from the test for Loss on drying to a suitable flask fitted with a reflux condenser, add 5 mL of a mixture of methanol and water (1 in 10), and heat under reflux for 15 minutes. While still hot, filter the contents of the flask with the aid of a vacuum. Rinse the flask and the test specimen with 2 mL of a mixture of methanol and water (2 in 100), and transfer the rinsings to the filter with the aid of a vacuum. Return the powdered Ginkgo to the flask, add 4 mL of a mixture of methanol and water (1 in 10), and repeat the extraction. After filtration, wash the residue of powdered Ginkgo twice with 1.5 mL of a mixture of methanol and water (2 in 100). Combine the filtrates, and transfer the combined filtrates (about 12 mL) to a solid-phase extraction column containing L1 packing with a sorbent mass-to-column volume ratio of 1000 mg per 3 mL or equivalent. [note—Initially pass 10 mL of methanol and then 10 mL of a mixture of methanol and water (2 in 100) through the column to condition it. Do not allow the column to dry.] Collect the eluate at the rate of 1 drop per second. Evaporate the eluate to dryness, and dissolve the residue in 2 mL of methanol.

Developing solvent system: a mixture of ethyl acetate and methyl acetate (1:1).

Procedure— Prepare a suitable thin-layer chromatographic plate coated with Adsorbent as follows. Immerse the plate for 20 seconds in a solution of sodium acetate in methanol containing 1

g per 10 mL. Allow the excess coating liquid to drip from the plate, and dry in a forced-air oven at 70°C for 30 minutes. Cool in a desiccator. Separately apply several 10-µL spots of the test solution to the impregnated plate, and allow the spots to air-dry. Develop the plate in the Developing solvent system in a chromatographic chamber without filter paper attached to the walls until the solvent front travels about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry in an oven at 105°C for 15 minutes. Spray the plate with acetic anhydride, and heat in an oven at 140°C for 25 minutes. Cool, and examine the plate under short- and long-wavelength UV light. [note—The compounds present in high concentrations may be visible in daylight as light brown spots.] The presence of terpene lactones in the test solution is shown by the following spots detected in the chromatogram at both the short and long wavelengths: bilobalide (R_F about 0.75), ginkgolide A (R_F about 0.68), ginkgolide B (R_F about 0.52), ginkgolide J (R_F about 0.39), and ginkgolide C (R_F about 0.27). Other spots of varying intensities also may be seen.

[Stems and other foreign organic matter](#) [561](#) : not more than 3.0% of stems and not more than 2.0% of other foreign organic matter.

[Pesticide residues](#) [561](#) : meets the requirements.

[Loss on drying](#) [731](#) — Dry 1.0 g of finely powdered Ginkgo at 105°C for 2 hours: it loses not more than 11.0% of its weight. Reserve the dried test specimen for use in Identification test B.

[Total ash](#) [561](#) : not more than 11.0%, determined on 1.0 g of finely powdered Ginkgo.

[Microbial enumeration](#) [2021](#) — It meets the requirements of the tests for absence of Salmonella species and Escherichia coli. The total aerobic bacterial count does not exceed 10⁵ cfu per g, the total combined molds and yeasts count does not exceed 10³ cfu per g, and the bile-tolerant Gram-negative bacteria does not exceed 10³ cfu per g.

Content of flavonol glycosides—

Extraction solvent— Prepare a mixture of alcohol, water, and hydrochloric acid (50:20:8).

Mobile phase— Prepare a mixture of methanol, water, and phosphoric acid (100:100:1). Make adjustments if necessary.

Standard solutions— Transfer accurately weighed quantities of USP Quercetin RS, kaempferol, and isorhamnetin to separate volumetric flasks, dissolve each in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain Standard solutions 1, 2, and 3 having known concentrations of 0.02, 0.02, and 0.005 mg per mL, respectively.

Test solution— Transfer about 1.0 g of Ginkgo, finely powdered and accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 78 mL of Extraction solvent, and reflux on a hot water bath for 135 minutes. [note—The solution will turn deep red. The color of the solution is not a definitive indication of reaction completeness.] Allow to cool at room temperature. Decant to a 100-mL volumetric flask. Add 20 mL of methanol to the 250-mL flask, and sonicate for 30 minutes. Filter, collect the filtrate in the 100-mL volumetric flask, wash the residue on the filter with methanol, collect the washing in the same 100-mL volumetric flask, dilute to volume, and mix.

Chromatographic system— The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph Standard solution 1, and record the peak responses as directed for Procedure: the relative retention times are about 1.0 for quercetin, 1.8 for kaempferol, and 2.0 for isorhamnetin; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 20 µL) of each of the Standard solutions and the Test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each flavonol glycoside in the portion of Ginkgo taken by the formula:

$$10(2.51)(C/W)(r_U/r_S)$$

in which 2.51 is the mean molecular mass factor to convert each analyte into flavonol glycoside with a mean molecular mass of 756.7; C is the concentration, in mg per mL, of [USP Quercetin RS](#) in Standard solution 1; W is the weight, in g, of Ginkgo taken to prepare the Test solution; r_U is the peak area for the relevant analyte obtained from the Test solution; and r_S is the peak area of USP Quercetin RS in Standard solution 1. Calculate the total percentage of flavonol glycosides by adding the individual percentages calculated.

Content of terpene lactones—

Solvent— Prepare a mixture of methanol and water (9:1).

Buffer solution— Dissolve 1.19 g of dibasic sodium phosphate and 8.25 g of monobasic potassium phosphate in 1000 mL of water, and adjust to a pH of about 5.8.

Diluent— prepare a mixture of methanol and water (1:1).

Solution A— Use filtered and degassed water.

Solution B— Use filtered and degassed methanol.

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary.

Standard solutions— Dissolve accurately weighed quantities of [USP Ginkgo Terpene Lactones RS](#) in Diluent, sonicating for a few minutes, and dilute with Diluent to obtain solutions having known concentrations of about 0.25, 0.5, 1.0, 2.0, and 4.0 mg per mL. Pass through a filter having a 0.45- μm or finer porosity.

Test solution— Transfer about 2.5 g of Ginkgo, accurately weighed, to a 30-mL glass centrifuge tube with screw cap and PTFE gasket. Add 10.0 mL of Solvent, seal the tube, and mix well on a vortex mixer. Heat in a water bath at 90°C for 30 minutes. Mix the hot suspension on a vortex mixer, and repeat the heating at 90°C for 30 minutes. Cool, centrifuge, transfer the supernatant to a flask, and return the residue to the glass tube. Repeat the extraction two more times, each time using 10.0 mL of Solvent. Combine the extracts, allow them to cool to room temperature, and evaporate to dryness under vacuum on a water bath maintained at 50°C. Add 10 mL of Buffer solution to the residue, and sonicate for 5 minutes. Quantitatively transfer the solution to a glass chromatographic tube filled with chromatographic siliceous earth capable of holding 20 mL of aqueous phase.* Rinse the beaker with two 5-mL portions of Buffer solution, and transfer the washings to the column. [note—Do not exceed 20 mL of total aqueous phase or the holding capacity of the chromatographic tube.] Allow the Buffer solution to be absorbed into the column. After 15 minutes, elute the column with 100 mL of ethyl acetate, collect the ethyl acetate solution, and evaporate to dryness under vacuum on a water bath maintained at 50 . Dissolve the residue in 10.0 mL of Diluent.

Chromatographic system— The liquid chromatograph is equipped with an evaporative light-scattering detector and a 4.6-mm \times 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 25 \pm 1 . [note—The

parameters of the detector are adjusted to achieve the best signal-to-noise ratio, according to manufacturer recommendations.] The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–23	75→52	25→48	linear gradient
23–28	52	48	isocratic
28–30	52→25	48→75	linear gradient
30–35	25→10	75→90	linear gradient
35–40	10→75	90→25	linear gradient
40–50	75	25	isocratic

Chromatograph the Standard solutions, and record the peak responses as directed for Procedure: the chromatograms obtained are similar to the Reference Chromatogram provided with [USP Ginkgo Terpene Lactones RS](#); and the relative standard deviation determined from the bilobalide peak for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 15 µL) of each of the Standard solutions and the Test solution into the chromatograph, record the chromatograms, and identify the peaks of the relevant analytes in the chromatogram of the Standard solution by comparison with the Reference Chromatogram. Measure the areas of the analyte peaks. Plot the logarithms of the relevant peak responses versus logarithms of concentrations, in mg per mL, of each analyte obtained from the Standard solutions, and determine the regression line using a least-squares analysis. The correlation coefficient for the regression line is not less than 0.995. From the graphs so obtained, determine the concentration, C, in mg per mL, of the relevant analyte in the Test solution. Separately calculate the percentages of bilobalide (C₁₅H₁₈O₈), ginkgolide A (C₂₀H₂₄O₉), ginkgolide B (C₂₀H₂₄O₁₀), and ginkgolide C (C₂₀H₂₄O₁₁) in the portion of Ginkgo taken by the formula:

$$1000(C/W)$$

in which W is the weight, in mg, of Ginkgo taken to prepare the Test solution. Calculate the total percentage of terpene lactones in the portion of Ginkgo taken by adding the percentages calculated for each analyte.