Evaluation of Commercial Ginseng Products

LAWRENCE E. LIBERTI and ARA DER MARDEROSIAN ˣ

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Abstract □ The variation in panaxoside content of roots and commercial products of *Panax ginseng* and *P. quinquefolium* was evaluated by a new spectrodensitometric TLC method. The method is rapid and applicable to all commercial products, and it has a relative standard deviation of 6.4%. Panaxoside patterns of slurry-filled capsules and root extracts most closely resembled those of whole roots. Tablets did not contain detectable panaxosides while teas and granules for infusion yielded only low concentrations. The wide variation among these products indicates the need for more rigid control.

Keyphrases □ Ginseng—panaxoside content of various commercial products, TLC analysis □ Panaxoside content—ginseng, various commercial products, TLC analysis □ TLC—analysis, panaxoside content of various commercial ginseng products □ *Panax ginseng* and *P. quinquefolium*—panaxoside content of various commercial products, TLC analysis

A resurgence in the use of folkloric medicine has prompted a return to traditional herbal remedies. Ginseng, *Panax quinquefolium* L. and *P. ginseng* C. A. Meyer (Araliaceae), is receiving much attention. Ginseng, whose characteristic taste is disagreeable, is available as the dried root, which is chewed, taken as tea, or made into an elixir. It is also available as capsules, tablets, instant teas, extracts, and a line of cosmetics. The popularity of ginseng is attributed to the "adaptogenic" effect (stress protective) of the saponin (panaxoside) content (1), which appears to exert an antistress and rehabilitative action in test animals. Ginseng is also heavily promoted as an aphrodisiac.

Ginseng is not included in the Generally Recognized as Safe (GRAS) list, nor has the government set guidelines for the manufacture and quality control of commercial ginseng preparations. Seizures of ginseng tablets by the Food and Drug Administration as an unsafe food additive when no regulations describe conditions for safe ingestion (2) press the need for standardizing ginseng products.

The TLC assay in this report was developed as a rapid screen for the evaluation of the panaxoside content of the hundreds of commercially available ginseng products.

BACKGROUND

Although ginseng has been used for centuries, the problem of evaluating commercial ginseng products is recent. Ginseng has not been an official compendial drug in the United States since the U.S. Dispensatory dropped it after noting that the "extraordinary properties and medical virtues formerly ascribed to ginseng had no other existence than in the imagination of the Chinese" (3). In light of both traditional effectiveness and recent experiments supporting the adaptogenic effect of the panaxosides (1), ginseng is included in several modern compendia.

Official compendia (4–6) that include ginseng do not evaluate ginseng root in terms of chemical constituents but by physical and microscopic characteristics. The Russian pharmacopeia (7) lists a "reaction for quality" of ginseng roots, involving a subjective evaluation of color reaction and precipitate formation over 24 hr following the addition of silver nitrate.

Only recently have more sophisticated attempts been made to evaluate the saponin content of ginseng roots and products. Nine saponins (panaxosides Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg2) are present in varying amounts in fresh ginseng roots, but their isolation with good recovery following recrystallization is difficult (8).

Table I—Determination of Recovery Rate (Theoretical Amount Present, 0.40 μg)

Sample	Amount Determined by TLC Method, μg	Recovery, %
Rb2	0.38	95
Rd	0.39	97
Rg1	0.38	95
	Average	96

Table II—Comparison of TLC and Spectrophotometric Methods

Sample	Total Milligrams of Panaxoside per 250-mg Sample: Spectrophotometric Method	TLC Method
1	3.0	3.3
7	2.2	1.9
10	None	None
13	1.6	1.7
15	None	None
16	0.5	None
21	1.0	1.0

A GLC method was reported for the evaluation of ginseng roots and extracts (9). The assay, based on the fact that the hydrolysis of the saponins yields panaxadiol, panaxatriol, and oleanolic acid genins, is incapable of quantitatively or qualitatively evaluating the individual panaxosides. A colorimetric reaction with vanillin–sulfuric acid was proposed for the spectrophotometric determination of total saponin content (10). Methods developed using rod-TLC (11) and countercurrent chromatography (12) for the quantitative and qualitative evaluation of individual panaxosides are reliable but not readily available for the large-scale screening of commercial products. Therefore, the following TLC method is proposed.

EXPERIMENTAL

Apparatus—A spectrodensitometer[1] was coupled to a density computer[2]. Plates[3] were scanned at 600 nm using a slit aperture of 2 cm and sensitivity of 0.4 absorbance unit.

Standard Solutions—Standard solutions of panaxosides Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg2 were each prepared by dissolving the panaxoside in methanol to yield a final concentration of 1 mg/10 ml. All stock solutions were refrigerated.

Detection Reagent—A solution of 1 ml of *p*-anisaldehyde and 1 ml of concentrated sulfuric acid diluted to 100 ml with methanol was prepared fresh daily. Plates were heated for 15 min at 130° following spraying.

Sample Solution Preparation—After the contents of the dosage form were weighed, 250 mg was transfered to a 10-ml flask to which 5 ml of water had been added. The solution was shaken for 30 min. The flask was then filled to 10 ml, and shaking was continued for an additional 30 min. The solution was filtered through a 70-mm paper.

The first 3–4 ml of filtrate was discarded. The next 5-ml aliquot was transferred to a 10-ml flask to which a 5-ml aliquot of water-saturated 1-butanol was added. The flask was shaken gently for 30 min. Samples were drawn from the 1-butanol layer and applied as 8-μl spots.

Plate Structure and TLC Assay—Each scored plate was spotted on alternate lanes. The first 10 lanes were used for the Beer's law calibration. Samples of 2, 3, 4, 5, and 8 μl of standard Re were spotted. Samples for analysis were spotted alternately in quadruplicate in the last eight lanes.

[1] Schoeffel model SD-3000.
[2] SDC-300.
[3] Silica gel G 250 μm (Analtech) activated at 130° for 15 min.

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Table III—Comparison of Individual and Total Panaxoside Content of Roots and Commercial Products

Sample[a]	Individual Panaxoside Concentration, mg									Total Panaxoside Concentration per 250-mg Sample, mg
	Ro	Rb1	Rb2	Rc	Rd	Re	Rf	Rg1	Rg2	
SA-1	—	0.79	—	0.58	0.50	0.29	0.53	—	0.57	3.26
SA-2	—	1.2	3.7	—	—	1.5	—	1.2	—	7.6
SA-3	—	1.8	—	2.2	0.38	0.67	1.8	—	—	6.85
SA-4	No detectable panaxosides									
SA-5	0.49	0.26	—	0.29	0.42	0.13	0.19	—	—	1.78
SA-6	0.36	0.80	0.18	0.32	—	—	0.27	—	—	1.93
SA-7	—	0.86	—	0.69	—	—	—	—	0.31	1.86
SA-8	—	0.30	—	—	0.77	—	0.32	—	—	1.39
SA-9	No detectable panaxosides									
SA-10	No detectable panaxosides									
SA-11	No detectable panaxosides									
SA-12	0.42	0.45	0.10	0.26	—	0.17	0.40	—	—	1.8
SA-13	0.42	0.22	—	0.44	0.31	0.10	0.17	—	—	1.66
SA-14	No detectable panaxosides									
SA-15	No detectable panaxosides									
SA-15a	—	0.15	0.16	0.16	0.10	0.10	0.30	—	—	0.97
SA-16	No detectable panaxosides									
SA-17	—	0.36	—	—	—	—	0.26	—	0.29	0.91
SA-18	No detectable panaxosides									
SA-19	0.33	0.27	—	—	0.27	—	—	—	—	0.87
SA-20	—	0.26	—	—	—	—	—	—	—	0.26
SA-21	—	0.45	—	—	—	0.58	—	—	—	1.03
SA-22	—	—	—	—	—	0.26	—	—	—	0.26
SA-23	—	—	—	—	—	0.60	—	—	—	0.60

[a] Samples 1–6 were roots, Samples 7 and 8 were slurry-filled soft gelatin capsules, Samples 9–11 were tablets, Samples 12 and 13 were dry-filled capsules, Samples 14–15a were extracts, Samples 16–22 were granules and powders for water infusion, and Sample 23 was ginseng-containing soap.

Plates were single developed to 15 cm in methanol–chloroform–1-butanol (1:1:1), dried, sprayed, heated, and scanned.

A Beer's law plot was developed for each plate by evaluating the area under the curve (*AUC*) of the five reference spots. Plot linearity was evaluated with a linear least-squares weighted fit program. The average *AUC* values for the four sample runs were used for the calculation of individual panaxoside concentrations.

Recovery Rate—A mixture of 5 ml each of Rb2, Rd, and Rg1 stock solutions was used. A 10-ml aliquot of water-saturated 1-butanol was added to the mixture, which was then shaken for 30 min. Samples were drawn from the 1-butanol layer. Lanes were spotted with 8-μl samples (equivalent to 0.4 μg of each panaxoside/8 μl). The plates were developed and scanned, and the amount recovered was determined (Table I).

Limits of Detection—A standard solution of Re was spotted on TLC plates in the following concentrations: 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 2.0, 3.0, 4.0, and 6.4 μg. Plates were developed and scanned, and plot linearity was evaluated as described previously.

Microscopic Evaluation—Each sample was observed under low and high powers for the presence of characteristic plant parts. Tablets were triturated lightly. All slides were cleared with chloral hydrate solution; teas and powders for infusion were suspended with a drop of water and observed before and after dissolution.

Comparison to Spectrophotometric Method—The spectrophotometric method of Hiai *et al.* (10) is a rapid means of evaluating total panaxoside content. To 0.1 ml of aqueous solutions of each sample, 0.5 ml of 8% vanillin in ethanol and 5 ml of 72% H_2SO_4 were added and mixed well in an ice water bath. The tubes were warmed at 60° for 10 min and then cooled in an ice bath. The absorbance of the reaction mixture was read[4] at 544 nm against a blank solution, and the amount present was determined by molar ratios.

RESULTS AND DISCUSSION

Limits of Detection—The range of detection remained linear in accordance with Beer's law from 0.1 to 4.0 μg. A change in the spectrodensitometer sensitivity from 0.4 to 1.0 unit dropped the lower detectable value to 0.4 μg and included the 6.4-μg reading. Since most samples yielded panaxosides in the range of 0.1 to 1.0 μg, all readings were performed at the higher sensitivity.

A 1-butanol transfer step was incorporated to avoid the detection of free sugars in sample extracts. In this manner, glucose, glucuronic acid, sucrose, rhamnose, and arabinose [present in whole root samples (13, 14)] were not detectable upon development with *p*-anisaldehyde. Reagents with greater specificity for triterpene steroids, such as antimony(III) chloride and chlorosulfonic acid, yielded less intense spots, which conformed poorly to Beer's law upon quantitation. The lower limit of detection using these reagents was only 0.8 μg.

Microscopic Evaluation—Each sample was scanned under polarized light at 100 and 430×. Root samples of *P. ginseng*[5] and *P. quinquefolium*[6], ground lightly in a mortar and pestle, yielded pale-yellow powders with large quantities of fragmented parenchyma and an almost equal amount of fiber groups of varying sizes. Occasional calcium oxalate crystals (7) were found along with fragments of bordered pitted vessels.

The commercial products varied widely in characteristic root inclusions. Samples 7, 12, 13, and 17 yielded large quantities of parenchyma and fiber fragments. Sample 17 yielded starch granules identified by reaction with iodine. Tablets did not display characteristic root parts. Teas consisted primarily of lyophilized powders, which yielded no characteristic fragments either before or following dissolution. Calcium oxalate crystals were not found in any commercial product.

Comparison of TLC and Spectrophotometric Methods (10)—An agreement was observed between these two methods (Table II). The spectrophotometric method is a useful means of estimating total saponin content, and the TLC method quantitates both individual panaxoside content and total saponin content. The spectrophotometric assay has the disadvantage of being time dependent because of the reaction of vanillin with sugars in the test solution. The 1-butanol transfer step cleaned the sample of interfering sugars while affording a 96% recovery of panaxosides from water solution. Plate color development remained unchanged for 45 min following removal from the oven.

Product Assay—Results of the TLC assays of 24 roots and products are presented in Table III. Dried roots of *P. ginseng* and *P. quinquefolium* yielded the highest panaxoside contents, ranging from 0.5 to 3.0% of dry root weight. Dried ginseng roots generally contain 2.5% panaxoside[7], a figure that varies with root age, method of preservation, and season of harvest. Sample 4, a 60-year-old herbarium specimen ravaged by insects, yielded no panaxosides.

Soft gelatin capsules containing slurries of ground root and those containing powdered root showed total panaxoside levels comparable to dried root samples. That these samples exhibited characteristic root inclusions by microscopic evaluation supports the conclusion that their contents most closely resemble ground ginseng root. These products yielded TLC patterns similar to ground roots.

The lack of detectable panaxosides in tablet samples along with a lack of identifiable root parts in the granulation indicates that these products

[4] Hitachi-Perkin Elmer 139 UV-visible spectrophotometer.

[5] Tatra Herb Co., Morrisville, PA 19067.

[6] Authentic samples were obtained from the herbarium, Philadelphia College of Pharmacy and Science, Philadelphia, Pa.

[7] "Ginseng," Inverni Della Beffa Inc., Milan, Italy.

may be adulterated or diluted during manufacture. When the ginseng powder is reduced to 40 or 60 mesh in the granulation, microscopic evaluation becomes less confirmatory. However, panaxoside levels should still be detected. The absence of detectable amounts of panaxosides requires further investigation into the manufacturing of ginseng tablets.

Granules and powders for the preparation of ginseng water infusions yielded panaxoside levels in the low region (0.1–0.4% of product weight).

Extracts yielded surprisingly low panaxoside levels, probably because of the relatively short shelflife of the opened extracts. The cosmetic tested yielded 0.3% panaxoside, within the recommended level of 0.25–0.5% for creams and rinses (15).

The TLC pattern of the roots can be correlated with the patterns of the powder-filled capsules and extracts and, to a lesser extent, the slurry-filled soft gelatin capsules. Most roots present at least four detectable panaxosides. Six-year-old roots show more extensive patterns and may yield up to nine panaxosides. All root samples tested yielded Rb1, while four of five yielded Rc, Re, and Rf. A similar pattern was seen with the capsule and extract products.

It is difficult to explain the tremendous variation among ginseng products. The wide range of total panaxoside content may be a function of the amount of ginseng root incorporated into the product. The lack of a consistent pattern of individual panaxosides in these products requires further clarification. The possibility of panaxoside loss during the manufacture of ginseng tablets is under investigation.

In view of the high prices of these products, there is a need for regulations regarding the control of commercial ginseng products.

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Use of a Nitrogen Detector for GLC Determination of Fluorouracil in Plasma during Single- and Combined-Agent Chemotherapy

ROSEMARY E. FINCH *x, MICHAEL R. BENDING ‡, and ARIEL F. LANT *

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Abstract □ A GLC assay for fluorouracil was developed and used to monitor plasma drug levels in patients on both single- and combined-agent chemotherapy. Fluorouracil is extracted from plasma, derivatized by flash methylation, and estimated using a thermionic nitrogen–phosphorus detector. The GLC determination was accurate at concentrations as low as 0.1 μg/ml of human plasma. Other drugs commonly used in combination with fluorouracil did not interfere with the assay.

Keyphrases □ Fluorouracil—nitrogen-detector GLC analysis in plasma □ GLC, nitrogen detector—analysis, fluorouracil in plasma □ Antineoplastic agents—fluorouracil, nitrogen-detector GLC analysis in plasma

Since its synthesis in 1957, fluorouracil has been used extensively as an antimetabolite in the treatment of cancer, particularly of the breast and GI tract (1). More recently, it has been widely used in multiple-agent chemotherapy (2, 3). Despite this continued usage, the clinical response is often inconsistent, and a variety of empirical dosage schedules are still employed. Therefore, to optimize drug schedules for individual patients, it would be useful to relate the time course of fluorouracil in plasma, both alone and in combination with other cytotoxic drugs, to clinical response.

Most methods for measuring fluorouracil employ GLC techniques (4–6) or assay by analysis of microbial kinetics (7). The present study describes a GLC method with nitrogen detection capable of measuring fluorouracil in combination with other cytotoxic agents without interference.

EXPERIMENTAL

Materials—Fluorouracil[1] was used both in the solid form and as a solution for injection. Thymine[2], the internal standard, was obtained as the anhydrous solid. Trimethylanilinium hydroxide[3], 0.2 *M* in methanol, was the derivatizing agent. Buffer salts[4] and solvents[4] were analytical reagent grade.

GLC Conditions—A gas chromatograph[5] equipped with a thermionic

[1] Roche Products Ltd., Welwyn Garden City, Herts., England.
[2] Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey, England.
[3] Pierce and Warriner (UK) Ltd., Chester, Cheshire, England.
[4] B.D.H. Chemicals Ltd., Poole, Dorset, England.
[5] Model F30, Series III, Perkin-Elmer Ltd., Beaconsfield, Bucks, England.

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