Supporting Information to:

An *in vitro* Evaluation of Cytochrome P450 Inhibition and P-Glycoprotein Interaction with Goldenseal, *Ginkgo biloba*, Grape Seed, Milk Thistle, and Ginseng Extracts and Their Constituents

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Preparation and Standardization of Goldenseal Extracts

Powdered goldenseal root was extracted with water or absolute ethanol. To prepare the aqueous extract, 5.0 g of root powder was steeped, with constant stirring, in water at 90 °C for 5 min. The resulting solution was filtered through diatomaceous earth (Sigma-Aldrich; St. Louis, MO, USA) and lyophilized. The ethanolic extract was prepared by stirring 5.13 g of goldenseal root powder in 500 mL of absolute ethanol for 24 h at room temperature. Following filtration, an additional 500 mL of ethanol was added to the residue and it was stirred for another 24 h at room temperature. The filtrates were combined and concentrated using a rotary evaporator.

Concentrations of the primary active constituents, berberine and hydrastine, in both aqueous and ethanolic extracts were determined by HPLC. The chromatographic system consisted of an Ace 5 C18 column and a mobile phase of 10 mM aqueous ammonium acetate (pH 4.85)/acetonitrile 78:22 at a flow rate of 1 mL/min. The alkaloids berberine and hydrastine were detected and quantitated with an Applied Biosystems 759A absorbance detector, using the integrated area of the absorbance at 267 nm. Standards were prepared using known concentrations of berberine (5 point standard curve, $r^2 = 0.9997$, LOQ = 12.5 ng/µL) and hydrastine (6 point standard curve, $r^2 = 0.9990$, LOQ = 12.5 ng/µL) and a standard curve was calculated by linear regression analysis.

Standardization of Ginkgo biloba Extract

Ginkgo biloba extract was analyzed for ginkgolide A, ginkgolide B, and bilobalide content by HPLC. The chromatographic system consisted of an ODS3 column and a mobile phase of water/methanol/THF 70:20:10 at a flow rate of 1 mL/min. The terpene lactones were detected using a Sedere Sedex 55 evaporative light scattering detector with a drift tube temperature of 50 °C and pressure of 2 bar. The identification of each of the lactones was verified by coelution with standards. Standards were prepared using known amounts of terpene lactones and a standard curve was calculated using linear regression analysis. The content of ginkgolide A (5 point standard curve, $r^2 = 0.9944$, LOQ = 0.75 µg), ginkgolide B (5 point standard curve, $r^2 = 0.9956$, LOQ = 0.75 g), and
bilobalide (5 point standard curve, \( r^2 = 0.9927 \), LOQ = 0.75 µg) was quantitated using regression variables and the integrated area of the light scattering response for each terpene lactone.

**Preparation and Standardization of Acid Hydrolyzed *Ginkgo biloba* Extract**

Flavone glycosides found in *Ginkgo biloba* extract were converted to aglycones by acid hydrolysis. Ginkgo extract (19.2 mg) was dissolved in 5 mL ethanol/water/HCl 50:20:8 and heated at 90 °C for 1 h. Hydrolyzed samples were analyzed for aglycones by HPLC. The chromatographic system consisted of an ACE 5 C18 column (MAC-MOD Analytical, Inc.; Chadds Ford, PA, USA) and a mobile phase of water/methanol 9:1 containing 0.25% formic acid at a flow rate of 1 mL/min. The flavonoids quercetin, kaempferol, and isorhamnetin were detected and quantitated using an Applied Biosystems 759A absorbance detector, using the integrated area of the absorbance at 270 nm.

**Standardization of Grape Seed Extract**

Grape seed extract was analyzed for catechin content by HPLC. The chromatographic system consisted of a Phenomenex Luna Phenyl-Hexyl column and a mobile phase that increased linearly from 5% acetonitrile in 0.1% aqueous phosphoric acid to 75% acetonitrile in 0.1% aqueous phosphoric acid over 60 min. The flow rate was 1 mL/min. Catechin was detected and quantitated with an Applied Biosystems 759A absorbance detector using the integrated area of the absorbance at 278 nm. Standards were prepared using known concentrations of catechin (7 point standard curve, \( r^2 = 0.9999 \), LOQ = 0.05 µg) and a standard curve was calculated by linear regression analysis.

**Standardization of Milk Thistle Extract**

Milk thistle extract was analyzed for silybin B content by HPLC. The chromatographic system consisted of a YMC-Pack ODS-A column and a mobile phase that increased linearly from 30% methanol in 0.1% aqueous phosphoric acid to 70% methanol in 0.1% aqueous phosphoric acid over 30 min. The flow rate was 1 mL/min. Silybin B was detected and quantitated with an Applied Biosystems 759A absorbance detector using the integrated area of the absorbance at 278 nm. Standards were prepared using known concentrations of silybin B (6 point standard curve, \( r^2 = 0.9949 \), LOQ = 0.5 µg) and a standard curve was calculated by linear regression analysis.
Standardization of Ginseng Extract

Ginseng extract was analyzed for the content of ginsenosides Rb₁, Rb₂, Rc, Rd, Re, Rf, and Rg₁ by HPLC. The chromatographic system consisted of a Phenomenex Luna C₁₈(2) column (4.6 X 150 mm, 5 µm) and a mobile phase of acetonitrile and water at a flow rate of 1 mL/min. The initial mobile phase condition of 20% acetonitrile was held constant for the first 35 min then linearly increased to 40% acetonitrile over 105 min. The individual ginsenosides were detected and quantitated, using peak height, with an Applied Biosystems 759A absorbance detector set at 205 nm. Standards were prepared using known concentrations of ginsenosides Rb₁ (8 point standard curve, \( r^2 = 0.9921 \), LOQ = 3.5 ng/µL), Rb₂ (8 point standard curve, \( r^2 = 0.9930 \), LOQ = 3.5 ng/µL), Rc (8 point standard curve, \( r^2 = 0.9936 \), LOQ = 3.5 ng/µL), Rd (8 point standard curve, \( r^2 = 0.9929 \), LOQ = 3.5 ng/µL), Re (7 point standard curve, \( r^2 = 0.9974 \), LOQ = 2.1 ng/µL), Rf (8 point standard curve, \( r^2 = 0.9948 \), LOQ = 3.5 ng/µL), and Rg₁ (8 point standard curve, \( r^2 = 0.9940 \), LOQ = 3.5 ng/µL) and a standard curve was calculated by linear regression analysis.

![HPLC profile of goldenseal ethanolic extract.](image)

Fig. 1S HPLC profile of goldenseal ethanolic extract.
Fig. 2S HPLC profile of goldenseal aqueous extract.

Fig. 3S HPLC profile of *Ginkgo biloba*.
Fig. 4S HPLC profile of grape seed extract.

Fig. 5S HPLC profile of milk thistle.
Fig. 6S HPLC profile of ginseng.