High-Performance Liquid Chromatography and Thin-Layer Chromatography Assays for Devil's Club (*Oplopanax horridus*)

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Abstract

Devil's root, *Oplopanax horridus*, is a widely used folk medicine in Alaska and British Columbia. The inner bark of the root and stem has been used to treat colds, cough, fever, and diabetes. The present study involves the development of high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC) methods to detect the presence of *trans*-nerolidol and sterols in the root bark. The HPLC and TLC analytical methods presented are suitable for the characterization and identification of *Oplopanax horridus*.

Introduction

Devil's root, *Oplopanax horridus* (Sm) Miq (*Araliaceae*), is currently and has been used in folk medicine by the native tribes of Alaska and British Columbia for centuries. The aqueous extract of the inner bark of the root is indicated for the treatment of colds, sore throats, fever, stomach trouble, rheumatoid arthritis, and diabetes. Recent studies report antifungal activities (1). Previous phytochemical investigation of *Oplopanax horridus* indicated the presence of several pharmacologically active compounds including equinopanacene and equinopanacol (2), saponins, glycerides and tannins (3), antimycobacterial polyynes (4,5), and steroid-related compounds (6).

Some of the aforementioned folk medicinal uses are supported by reported biological activity of certain constituents of *Oplopanax horridus* in the chemical literature. A mixture of *cis*- and *trans*-nerolidol was recently shown to act as an inhibitory agent against azoxymethane-induced neoplasia of the large bowel in male F344 rats (7). The rats were fed a diet that contained a mixture of *cis*- and *trans*-nerolidol. Nerolidol was also found to have activity as a sedative and spasmolytic in mice (8). Stigmasterol and beta-sitosterol, respectively, have antirheumatic and anticholesteremic properties (9). The biological properties of these chemical constituents were substantiated by articles found in *Chemical Abstracts* and *Medline*.

As the root bark of *Oplopanax horridus* develops from an herbal traditional medicine, it is important to develop a quality control procedure for this product. This study focuses on the development of high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC) methods to analyze important sterols and related compounds in the root bark. These compounds are used as markers for the characterization and identification, as well as verification, of the medicinal qualities of *Oplopanax horridus*.

Experimental

Materials and reagents

Reference samples of beta-sitosterol and stigmasterol were obtained from Sigma Chemical (St. Louis, MO), and *trans*-nerolidol was obtained from Aldrich Chemical (Milwaukee, WI). HPLC-grade acetonitrile and reagent-grade methanol, ethyl acetate, hexane, and acetone were obtained from Fisher Scientific (Hampton, NH). Purified water was obtained by filtration through an E-pure Alltech system (Deerfield, IL). Vanillin, ethanol, and sulfuric acid were reagent grade. Analytical and preparatory TLC were preformed on precoated plates (silica gel 60, F-254, Analtech, Newark, DE).

Plant material and extraction

Oplopanax horridus was authenticated at the University of the Sciences (Philadelphia, PA). Lyophilized root bark (5.0 g) was ground and extracted overnight in 50 mL MeOH–H_2O (80:20). The extract was filtered, and the volume was adjusted to 50 mL and used for HPLC analysis. A portion of

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the original extract was concentrated on a flash evaporator. The concentrated extract was used for TLC analyses.

TLC

Extract of the root bark was applied to silica gel GF TLC plates (20 cm × 20 cm × 250 µm) and developed in hexane–ethyl acetate (1:1). Visualization of spots was afforded by spraying developed plates with 5% ethanolic H_2SO_4, followed immediately by 1% vanillin in ethanol. Spots developed after approximately a 10-min heating at 80–100°C. Separated fractions for analysis were obtained through preparative TLC on silica gel plates (20 cm × 20 cm × 1500 µm). Reference spots visualized with ethanolic vanillin-sulfuric acid as previously mentioned were used as markers. The spots were scraped from the plate, and the silica gel was extracted overnight in methanol; the extracts were concentrated in a flash evaporator.

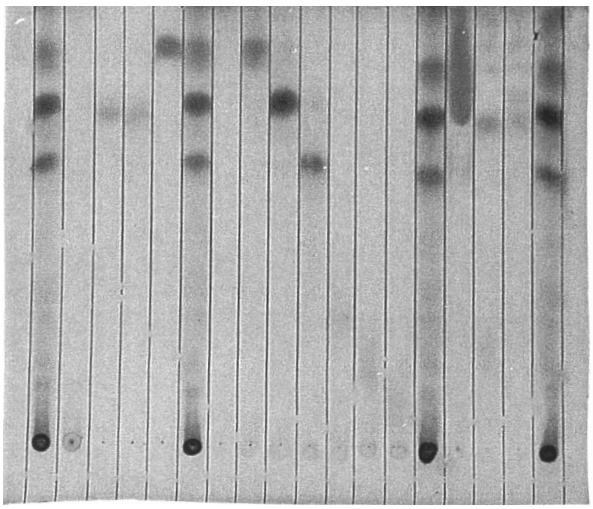

Figure 1. TLC of *Oplopanax horridus* and constituents: hexane–ethyl acetate (1:1). Visualization of bands with 5% ethanolic H_2SO_4–1% ethanolic vanillin, followed by heating at 80–100°C for 10 min. *Oplopanax horridus* extract: lanes 1, 6, 14, 18; beta-sitosterol: lane 3; cholesterol: lane 4; nerolidol: lane 5; fractions obtained through preparative TLC: lanes 7–13.

Table I. TLC R_F Values and Colors for Constituents of *Oplopanax horridus*

R_F value	Spot color
0.13	violet
0.19	gold
0.29	gray-green
0.37	violet
0.43	faint olive
0.46	dark olive
0.63	dark olive
0.87	dark olive
0.98	faint gray

HPLC

Reversed-phase HPLC analyses were conducted using a Rainin Rabbit HP (Varian, Walnut Creek, CA) with a Gilson Datamaster Model 620 HPLC system (Middleton, WI) with a Zorbax 300SB-C_{18} 5-µm column (250- × 4.6-mm i.d.) (Agilent, Palo Alto, CA) using absorbance detection at 202 nm with a

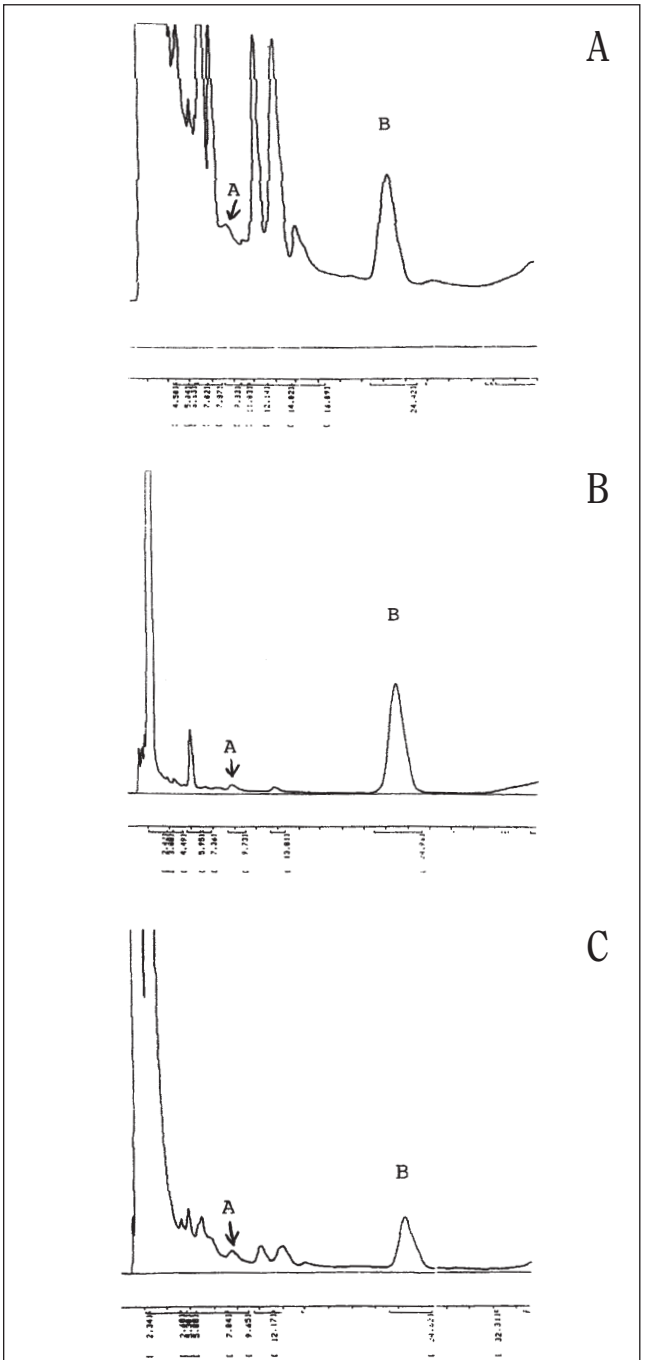

Figure 2. (A) HPLC chromatogram of the extracted solution of *Oplopanax horridus*: stigmasterol (labeled as A), retention time 9.3 min and *trans*-nerolidol (labeled as B), retention time 24.4 min. (B) HPLC chromatogram of stigmasterol (labeled as A), retention time 9.7 min and *trans*-nerolidol (labeled as B), retention time 25.0 min. (C) HPLC chromatogram of *Oplopanax horridus* extract that had been mixed with a standard solution of stigmasterol–*trans*-nerolidol (1:10). Stigmasterol (labeled as A), retention time 9.7 min and *trans*-nerolidol (labeled as B), retention time 24.6 min.

variable wavelength UV–vis detector. The mobile phase consisted initially of 60% acetonitrile–40% water for 30 min, followed by a gradient from 40% to 100% acetonitrile from 30 to 50 min and 100% acetonitrile from 50 to 67.5 min. The flow rate was 1.3 mL/min at a pressure of 2600 psi, and the injection volume was 10 µL. The samples were directly injected into the HPLC. A standard solution of stigmasterol and *trans*-nerolidol was prepared by dissolving 30.5 mg of stigmasterol and 0.63 mg of *trans*-nerolidol in 10 mL of ethylacetate.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analyses were conducted on a Saturn GC–MS instrument (Varian) with a mass selective detector. Samples of 1 µL were injected onto a Varian VF-5ms column (5% phenyl–95% dimethylpolysiloxane) with a 30-m length and 0.25-mm i.d. The following conditions were used for GC–MS analysis: initial temperature of 50ºC for 5 min, temperature increase of 20ºC/min until a temperature of 290ºC was realized, and a final time of 26 min at 290ºC. The detector temperature was 150ºC. The *trans*-nerolidol standard solution was prepared by dissolving 0.22 g *trans*-nerolidol in 10 mL reagent-grade acetone.

NMR

Carbon-13 NMR spectra were recorded at 75.48 MHz on a General Electric QE-300 spectrometer (Boston, MA). ^{1}H NMR spectra were recorded at 310.15 MHz on a General Electric QE-300 spectrometer. Chemical shifts were recorded in delta units from the internal standard of tetramethylsilane in chloroform-D.

Results and Discussion

TLC

TLC of *Oplopanax horridus* extract yielded a characteristic pattern of spots when treated with vanillin-sulfuric acid spray reagent (Figure 1). Nine different spots could be reliably distinguished on the TLC plates with colors and R_F values as indicated in Table I. The dark olive spot at R_F 0.87 corresponds with the migration distance and spot color obtained with authentic nerolidol, whereas the dark olive spot at R_F 0.75 corresponds with the migration distance and spot color obtained with authentic beta-sitosterol. The presence of nerolidol and beta-sitosterol were also confirmed by HPLC and GC–MS analyses. Additional structural proof of *trans*-nerolidol was afforded by comparison of ^{1}H and ^{13}C NMR of *trans*-nerolidol with an authentic sample from Aldrich Chemical. Individual fractions obtained by preparative TLC were reassayed to verify the isolation of spots before proceeding to HPLC and GC–MS analyses. The sterol, cholesterol (R_F 0.75), was a reference compound that is not present in *Oplopanax horridus*.

HPLC

The HPLC chromatogram of *Oplopanax horridus* extract indicated the presence of the sterol stigmasterol and *trans*-nerolidol (Figure 2A). Stigmasterol had a retention time of 9.3 min, and *trans*-nerolidol had a retention time of 24.4 min. The HPLC chromatogram of the standards stigmasterol and *trans*-nerolidol is given in Figure 2B. The HPLC chromatogram of *Oplopanax horridus* extract, which had been mixed with the standards stigmasterol and *trans*-nerolidol, is given in Figure 2C. As is indicated in Figure 2C, the retention times of the standards stigmasterol and *trans*-nerolidol are identical with these constituents of *Oplopanax horridus*.

GC–MS

GC–MS analysis of an HPLC fraction containing *trans*-nerolidol confirmed the presence of *trans*-nerolidol in the *Oplopanax horridus* extract (Figure 3A). The GC retention time of *trans*-nerolidol from the HPLC fraction of the *Oplopanax horridus* extract was 11.6 min. An authentic sample of *trans*-nerolidol also had a GC retention time of 11.6 min (Figure 3B). The MS fragmentation patterns of *trans*-nerolidol in *Oplopanax horridus* and an authentic *trans*-nerolidol sample were similar (Figure 4A and B).

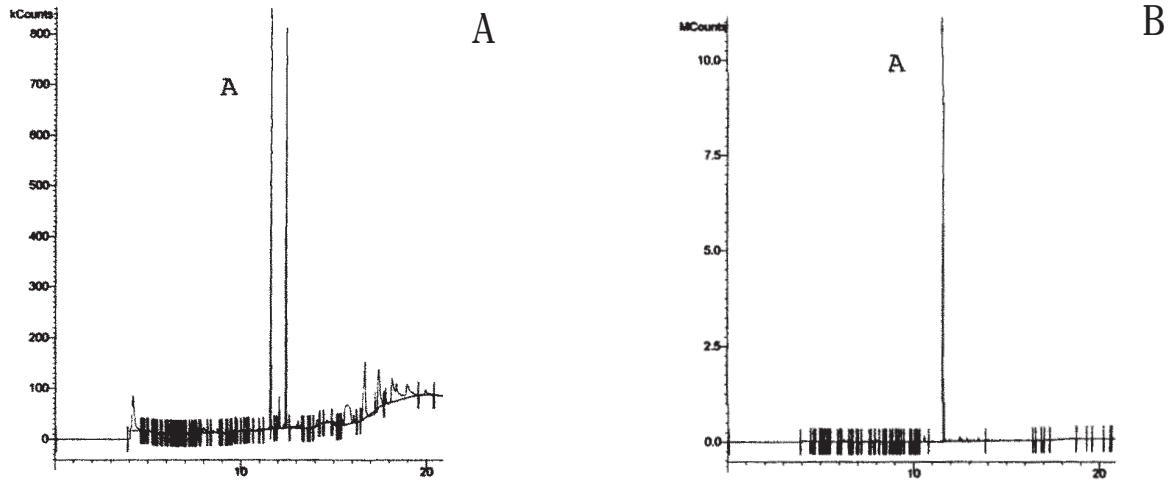

Figure 3. (A) GC–MS chromatogram of HPLC fraction of extracted solution of *Oplopanax horridus*: *trans*-nerolidol (labeled as A), retention time 11.6 min. (B) GC–MS chromatogram of *trans*-nerolidol standard (labeled as A), retention time 11.6 min.

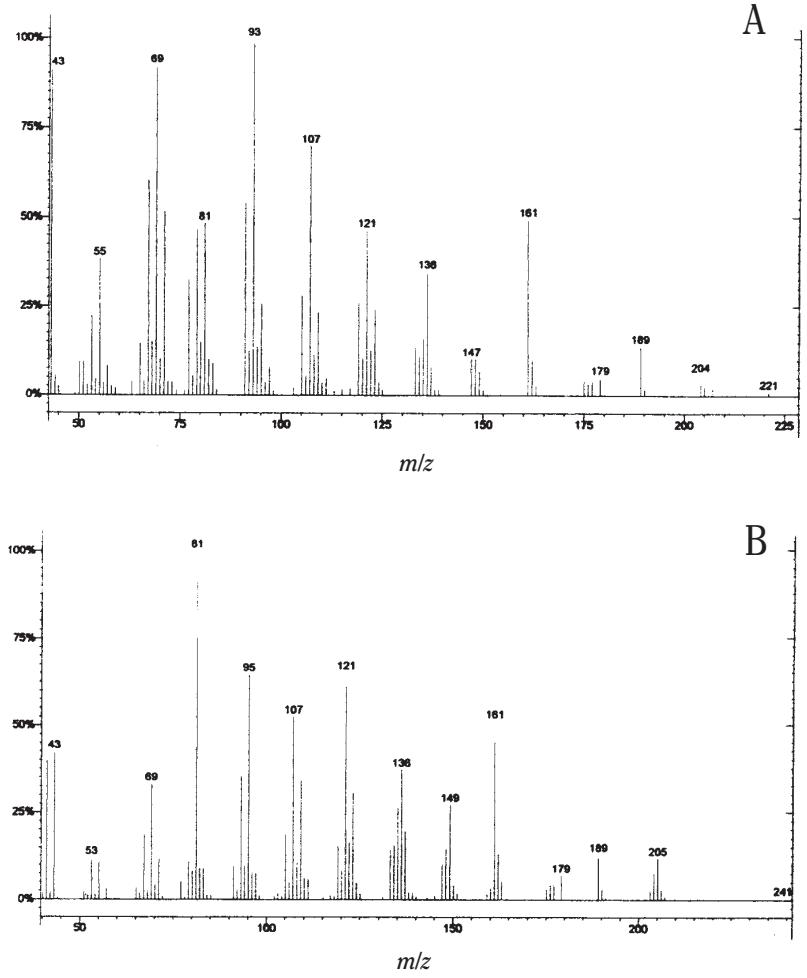

Figure 4. (A) MS of *trans*-nerolidol from an HPLC fraction of extracted solution of *Oplopanax horridus*: *trans*-nerolidol, retention time 11.6 min. (B) MS of *trans*-nerolidol standard, retention time 11.6 min.

Conclusion

In conclusion, the TLC and HPLC procedures described herein allow for the identification of samples of *Oplopanax horridus*. The traditional uses of *Oplopanax horridus* have been supported by the identification of compounds in the plant that have biological activities that are consistent with ethnobotanical uses of this species. This is another confirmation of the value of ethnobotanical knowledge.

Acknowledgments

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