Anti-Inflammatory Components of Mushroom Extract on SUPELCOSIL ABZ⁺Plus HPLC Columns

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Ganoderma lucidum, a mushroom long used in the East for treating a broad range of disorders, contains numerous pharmacologically active molecules, including an exceptionally diverse family of oxygenated triterpenes. The authors of this contributed article have determined that extracts of G. lucidum possess potent anti-inflammatory properties. They present some of their initial observations on the biological activity and chromatography of these acids. Standard reversed-phase fractionation does not resolve the hundreds of structural variants of ganoderic and lucidenic acids produced by the mushroom. However, semi-preparative C18 chromatography followed by sub-fractionation on a SUPELCOSIL ABZ⁺Plus column separates, to a significant degree, many of the acids.

Anti-inflammatory agents currently on the market have serious side effects that significantly limit their usefulness. For example, gastropathy caused by aspirin-like drugs accounts for at least 70,000 hospitalizations in the U.S. each year, and an estimated 7,000 to 20,000 deaths. Furthermore, aspirin-like compounds provide only symptomatic relief and do not treat underlying pathology. Steroids, on the other hand, frequently cause suppression of pituitary-adrenal function and seriously disturb fluid and electrolyte balance. In children, steroid use can prevent growth and can even cause death.

Ganoderma lucidum and preparations of this mushroom have a long history of use throughout the East as traditional medicines in the treatment of a broad range of disorders (1). More recently it has been found that this mushroom contains a number of pharmacologically active molecules: anti-tumor, hypoglycemic, and anti-fibrotic polysaccharides (2-4), an immunomodulatory protein (5), chemoprotective principles (6), a unique inhibitor of platelet aggregation (7), and an exceptionally diverse family of oxygenated triterpenes that is comprised predominantly of ganoderic and lucidenic acids (8) (Figure A).

Amelioration of the pain and inflammation associated with arthritis has historically been attributed to preparations of G. lucidum (9). We have determined that extracts rich in ganoderic and lucidenic acids, prepared from various portions of G. lucidum, possess potent systemic and topical anti-inflammatory properties (10). In this report, we present some of our initial observations on the biological activity of G. lucidum extracts, as well as the results of preliminary chromatographic analysis.

Electrospray ionization mass spectra were acquired on a Finnigan-MAT SSQ700 quadrupole mass spectrometer fitted with an Analytica of Branford electrospray interface. Samples were introduced in 70% aqueous acetonitrile/0.5% acetic acid solution at a flow rate of 1µL/min, with an electrospray voltage of -3.5kV.

Semi-preparative-scale liquid chromatographic fractionations were performed with a Milton Roy ternary gradient HPLC system, using a 10 x 250mm octadecyl column. A non-linear stepped gradient consisting of water/acetonitrile/5% aqueous acetic acid running 60 minutes at 2mL/min was used to effect separations; 1-min fractions were collected from 9 to 55 minutes. The fractions were lyophilized and resuspended in 70% aqueous acetonitrile for further chromatographic and mass spectrometric analyses. The fractions were sub-fractionated on an analytical scale using a Waters HPLC system with a 4.6 x 250mm SUPELCOSIL™ ABZ⁺Plus mixed functionality column and a nonlinear binary gradient.
Figure B. Anti-Inflammatory Activity of Extracts of
*Ganoderma lucidum* Pore Powder

### Biological Activity

In view of the tremendous structural heterogeneity of the acidic triterpenes produced by *G. lucidum* and the effort required to separate sufficient quantities of pure ganoderic acids for controlled testing of *in vivo* activity, for our preliminary investigation we assessed the anti-inflammatory efficacy of crude water and ethyl acetate extracts of the mushroom. For our *in vivo* assay, mice were treated either orally or topically with water and ethyl acetate extracts of *G. lucidum*. The ability of these extracts to alleviate the swelling produced by an irritant, croton oil, was compared to the efficacy of one of the most commonly used topical anti-inflammatory drugs, hydrocortisone. Figure B summarizes the results of these experiments.

It is clear that orally administered ethyl acetate and water extracts of *G. lucidum* have significant anti-inflammatory activity that extends over a longer period of time than does hydrocortisone. Moreover, topical application of the ethyl acetate extract results in significant anti-inflammatory activity that compares favorably with the activity of hydrocortisone. The water extract, on the other hand, has little or no topical effect.

The mice used in this study were sacrificed at the end of each experiment and the weight of the thymus in each animal was assessed. None of the animals in the irritant alone (control), water extract, and ethyl acetate extract groups exhibited a thymus weight significantly lower than normal. The animals treated with hydrocortisone, however, showed significant thymic involution, a toxicity associated with corticosteroid treatment. Thus, extracts of *G. lucidum* possess significant anti-inflammatory activity, and do not appear to exhibit the unwanted side effects encountered with corticosteroids. On the basis of these preliminary results for anti-inflammatory efficacy, we have now begun screening semi-preparative HPLC fractions for *in vivo* activity, thus allowing us to proceed more directly to the active components.

### HPLC Fractionation

Analysis of the ganoderic acids of *G. lucidum* is complicated by the exceedingly large number of ganoderic and lucidenic acids produced by the mushroom. Standard separation techniques relying on C18 reversed-phase fractionation are not equal to the task of resolving the hundreds of structural variants that are present. However, if semi-preparative C18 chromatography is followed by sub-fractionation using Supelco’s new mixed function ABZ+Plus column, it is possible to separate, to a significant degree, a large number of the ganoderic acids. Figure C shows an HPLC chromatogram of the 37-minute fraction collected during semi-preparative C18 separation and reinjected onto the semi-preparative column for further separation under the same conditions. Immediately apparent is the poor resolution of the components. Figure C also illustrates fractionation of the same sample by the ABZ+Plus column. Even though resolution is still not complete, we are able to resolve a much larger number of components.

*Oral Administration: 1 mL test solution administered by gavage 1 hr before application of irritant. Water extract dissolved in water, ethyl acetate extract suspended in 1% aqueous methyl cellulose, control group administered 1 mL water. *Statistical significance of difference from control group: p<0.001.*

*Topical Administration: Each test material dissolved in pyridine/water/diethyl ether (4:1:5) and applied to same location as irritant, 30 min after treatment with irritant. Control group treated with irritant alone. *Statistical significance of difference from control group: p<0.0001.*
The eluate from the ABZ+Plus column was collected in 1-min fractions and analyzed by direct infusion ESI-MS. The results of these analyses confirmed that the ABZ+Plus column effected considerable separation of the components that eluted together from the C18 column. Thus, the use of two columns of differing selectivity has allowed us to partially untangle the triterpene products of *G. lucidum*.

Our data show that the extracts of *G. lucidum* possess significant anti-inflammatory activity. While it is premature at this stage of the investigation to assign a rank order of potency to all of the members of this complex mixture of acidic triterpenes, our preliminary studies suggest that relatively minor structural differences among the components lead to measurable differences in anti-inflammatory potency. As we improve our abilities to isolate and characterize individual ganoderic acids, we expect to be able to deduce comprehensive structure-activity relationships for these molecules. This effort will be important not only for elucidating the mechanism of action of these molecules, but also for future analog drug development.

**References**


References not available from Supelco.

**Acknowledgments**

The authors wish to convey their appreciation to Lone Star Growers (San Antonio, Texas) and the Il Yang Pharmaceutical Company, Ltd. (Seoul, Korea) for their support of this investigation. We are also grateful to Tracy Ascah and Supelco for generously donating the SUPELCOSIL ABZ+Plus HPLC columns, which have been instrumental in resolving the tremendously complex mixtures of ganoderic acids.

**Ed. note**

We have not included the authors’ mass spectra, due to space constraints. Readers interested in additional details of this investigation are invited to contact the authors at Department of Biochemistry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7760 USA.

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