



Natural Product Purification: Bioactive trans-Cinnamaldehyde from Zingiberaceae Extract for Use as a Skin Lightening Agent

Application Note PHA0213

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Keywords

Cosmetics, Natural Products, Zingiberaceae, trans-Cinnamaldehyde, GX-281 Preparative Purification System, Biologically Active Compounds, High Pressure Liquid Chromatography (HPLC)



Introduction

Natural products are a common source of biologically active pharmaceutical compounds. Research is often performed to purify and isolate biologically active compounds from their natural sources for characterization and identification. Purified compounds that are identified can be used for further therapeutic research on a chronic condition or illness. This application focuses on the isolation of a skin lightening agent, trans-Cinnamaldehyde, purified with the Gilson GX-281 Preparative Purification System from the Zingiberaceae extract ; a perennial herb from the ginger family (see Figure 1). This perennial herb is used in the treatment of various skin disorders (1).



Figure 1:
Zingiber officinale or common,
Canton ginger
(source: www.britannica.com)

Standard laboratory techniques were used throughout to simplify the natural product purification process. Prior to isolation and purification, the crude sample extract was fractionated using LC-IT-TOF-MS in preparation for the bioactivity study (see Figure 2). All fractions and the crude were analyzed *in-vitro* (process and results are not shown in this application).



Materials & Methods

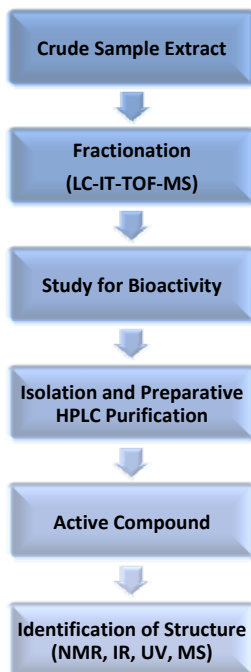


Figure 2: Methodologies for Purification and Identification of trans-Cinnamaldehyde

Sample Preparation Prior to Isolation and Preparative HPLC Purification

- Freeze dried Zingiberaceae aqueous extract was dissolved in a 50:50 water:methanol solution.
- Following centrifugation, a 150 mg (2 mL) of the supernatant was injected.

Isolation and Preparative HPLC Purification Method and Materials

- Column: Waters X-terra RPC-18, 19x30 mm, 5 μ m
- Mobile phase gradient: 0% B to 100% B @ 12 mL/min
 - A: Water + 0.1% formic acid
 - B: Acetonitrile + 0.1% formic acid
- Injection volume:
 - 2 mL
- UV detection:
 - 210 and 350 nm
- Fraction collection:
 - 0.5 min/ fraction with 6 mL/tube fraction
- Purification software: Gilson TRILUTION® LC



Results

Purification of trans-Cinnamaldehyde was performed from the extracted Zingiberaceae supernatant using the Gilson GX-281 Preparative Purification System (see Figure 3), shortening the process of isolation from other compounds by using common laboratory techniques. A total of 16 fractions were collected and purification yield results of these collected fractions A-P were calculated. Fraction D contained the purified trans-Cinnamaldehyde peak (see Figure 4). Following isolation, structure confirmation of trans-Cinnamaldehyde was performed (see Figure 2).

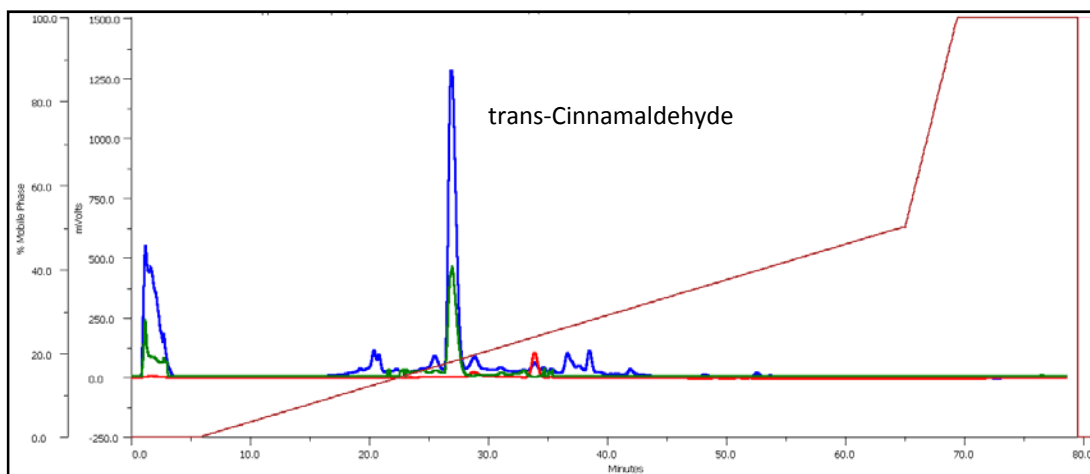


Figure 3: Purification of trans-Cinnamaldehyde from Zingiberaceae Extract

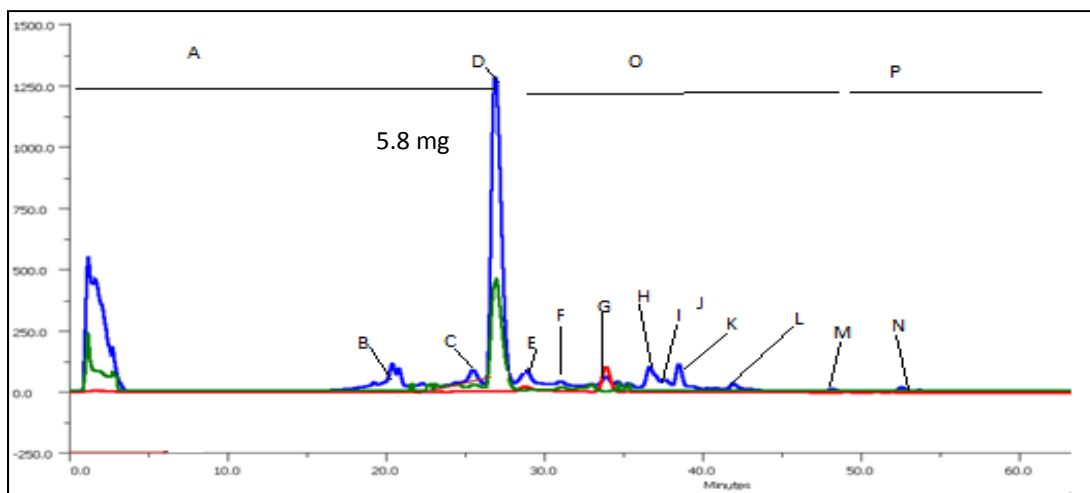


Figure 4: Collected 16 Fractions and Calculated Yield of trans-Cinnamaldehyde



Summary

Preparative HPLC purification has been successfully performed from the Zingiberaceae extract using the GX-281 Preparative Purification System to isolate trans-Cinnamaldehyde and quantify its yield. Using common laboratory techniques, scale-up to preparative purification resulted in the identification of the main natural bioactive compound, trans-Cinnamaldehyde from the 15 other compounds present in the herb extract. The isolation of these other 15 natural compounds is important for future research. As a result of this study and further investigation into identification and biological activity of natural compounds, therapeutic treatment of multiple skin disorders is possible. Using preparative purification, a sufficient quantity of trans-Cinnamaldehyde was purified from Zingiberaceae extract for this research.

References

1. Ding, H.Y., Lin, H.C., Chang, T. S. (2009). Tyrosinase inhibitors isolated from the roots of *Paeonia suffruticosa*. J. Cosmet. Sci., 60: 347-352.