BIOACTIVITY GUIDED SEPARATION OF RAW TURMERIC (*Curcuma longa*)

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ABSTRACT

The powdered dry rhizome of the plant *Curcuma longa*, commonly known as turmeric, has been used for centuries as a traditional medicine to treat inflammatory diseases. Identification and characterization of the active compounds of turmeric is subjective and very few official methods of analysis are available in the literature. In order to test the anti-inflammatory activity, an *in vitro* test system has been established. HL-60 cells were differentiated and exposed to lipopolysaccharide (LPS) from *E.coli* (1 µg/ml) in the presence or absence of botanical compounds for 24 hrs. Supernatants were collected and analyzed for the production of tumor necrosis factor alpha (TNF-α) and prostaglandin E_2 (PGE_2) by standard ELISA assays. In order to verify the amount and identity of active components in the raw turmeric samples, analytical and preparative HPLC methods were developed. Bulk raw powdered turmeric rhizomes were obtained from two different commercial sources. These samples were subjected to various aqueous and organic extractions and the resulting fractions were analyzed by HPLC. None of the aqueous extracts expressed biological activity, but the crude organic extracts were capable of inhibiting LPS induced TNF-α (IC_{50} = 15 and 25 µg/ml) and PGE_2 (IC_{50} = 1 µg/ml) production. One of the crude organic extracts was subjected to further separation using preparative HPLC and each of the resulting ten fractions was tested as described previously. These fractions had differing biological activity, ranging from no activity to IC_{50} < 1 µg/ml. Additional analytical methods were developed to further separate the most active fractions to test for anti-inflammatory activity.

INTRODUCTION

Demand for botanical products has grown dramatically over the past ten years, and markets are still robust. More than 120 million Americans try herbs, vitamins and dietary supplements to treat a variety of illnesses. Turmeric, the powdered rhizome of the herb *Curcuma longa* is used extensively in curries and mustards as a coloring and flavoring agent. In the Ayurvedic medicine turmeric has traditionally been used as a treatment for inflammation, skin wounds and tumors [1]. In preclinical animal studies turmeric has shown anti-inflammatory, cancer chemopreventive and anti-neoplastic properties [2].

EXPERIMENTAL PROCEDURES

- Various reversed phase HPLC methods were developed to separate and analyze the raw turmeric sample and its fractions
- Agilent 1100 preparative/purification system (preparative gradient pumps, multi-wavelength detector and sampler/fraction collector) was used
- Signals were observed at 425 and 250 nm
- Fractions were tested for ability to inhibit LPS-induced production of TNF-α and PGE2 in HL-60 cells

CONCLUSION

- The initial separation and further subfractionation of the complex turmeric mixture was achieved
- The resulting fractions had different biological activity
- In the subfractions tested, the inhibition of LPS induced TNF-α and PGE2 production was weaker than in the original parent fraction .

REFERENCES

[1] Ammon, H.P.T., and Wahl, M.A.: *Planta Med.,* **57,** 1-7 (1991)

[2] Kelloff, G.J *et al.*: *Cell Biochem.,* **63,** 54-71 (1996)

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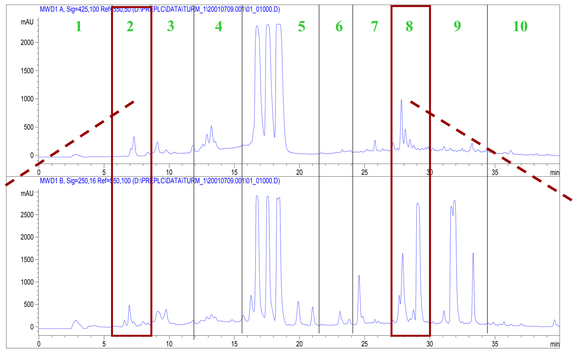
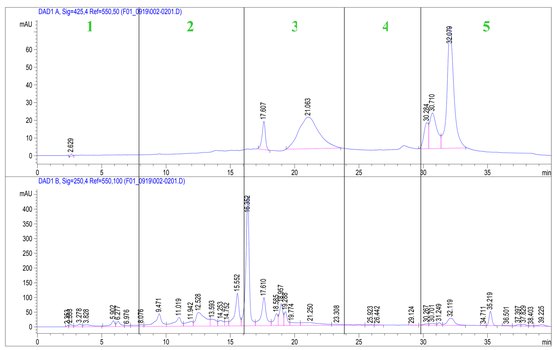
RESULTS

PREPARATIVE SEPARATION OF T1-1-F0 SAMPLE



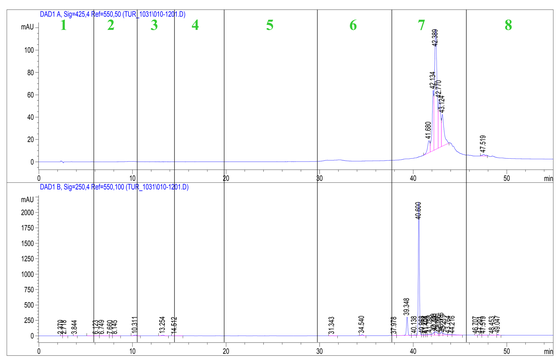
- Ten fractions were obtained
- The bioactivity of each fraction was determined

PREPARATIVE SEPARATION OF FRACTION 2



- Five fractions were obtained
- The bioactivity of each fraction was determined

PREPARATIVE SEPARATION OF FRACTION 8



- Eight fractions were obtained
- The bioactivity of each fraction was determined

INHIBITION OF LPS INDUCED TNF-α AND PGE$_2$ PRODUCTION

	IC (µg/ml)									
Fraction	1	2	3	4	5	6	7	8	9	10
TNF-α	29.2	6.50	5.8	11.7	18.9	39.4	-	26.7	25.6	-
PGE_2	2.2	4.7	3.5	1.0	0.9	3.7	7.5	1.7	6.3	6.3

INHIBITION OF LPS INDUCED TNF-α AND PGE$_2$ PRODUCTION

	IC (µg/ml)				
Subfraction	1	2	3	4	5
TNF-α	-	-	31.9	-	-
PGE_2	43.5	9.4	14.5	25.6	22.2

INHIBITION OF LPS INDUCED TNF-α AND PGE$_2$ PRODUCTION

	IC (µg/ml)							
Subfraction	1	2	3	4	5	6	7	8
TNF-α	-	-	33.7	-	-	-	-	-
PGE_2	-	35.4	9.6	-	8.8	16.2	7.4	-