

## Supporting Information

### **Comparative Study of Chromatographic Medium-Associated Mass and Potential Antitumor Activity Loss with Bioactive Extracts**

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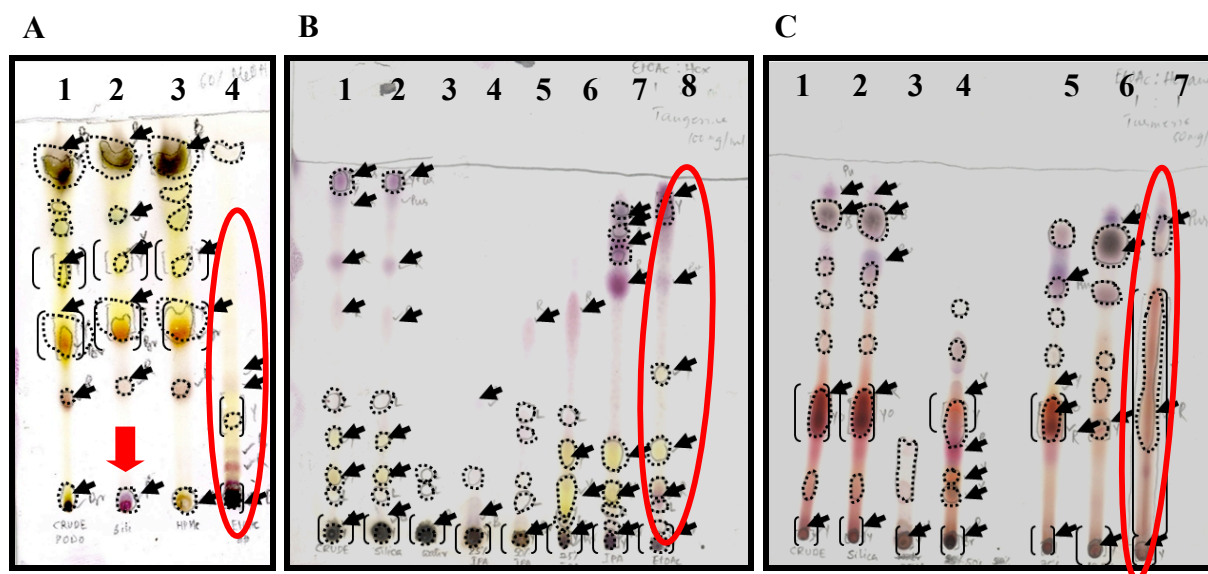
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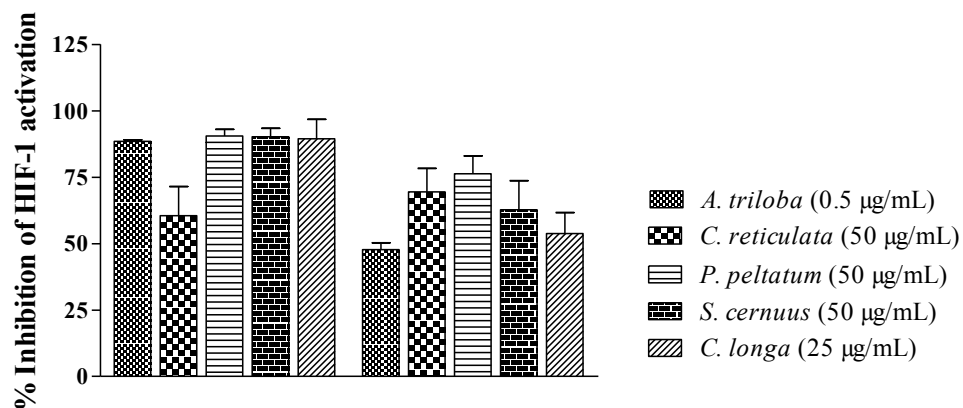
**Figure S1. TLC analysis of the crude extracts and fractions exposed to elution with chromatographic media.**

**(A)** TLC analysis of the *Podophyllum peltatum* crude extract exposed to elution with various chromatographic media. Notable chemical alterations in (Red arrow) and active phytochemicals retained in EtOAc eluent (solid circle); **1** – Crude *Po. peltatum* extract, **2** – Combined *Po. peltatum* fractions from a Si gel column, **3** – Combined water-MeOH fractions of *Po. peltatum* from a HP20SS column and **4** – EtOAc wash of *Po. peltatum* from a HP20SS columns after water-MeOH elution.  $C_{18}$  reversed-phase TLC eluted with 60% MeOH in water;

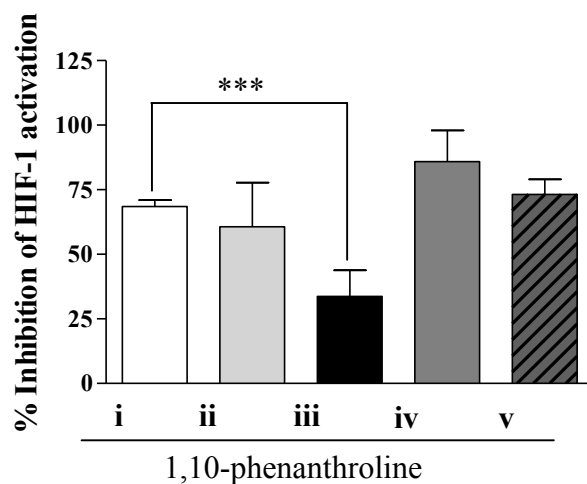
**(B)** TLC analysis of the *Citrus reticulata* crude extract and fractions exposed to elution with various chromatographic media. **1** – Crude *Ci. reticulata* extract, **2** – Combined *Ci. reticulata* fractions from a Si gel column, **3** – Water fraction of *Ci. reticulata* extract from a HP20SS column, **4** – Isopropanol-water (1:3) fraction of *Ci. reticulata* extract from a HP20SS column, **5** – Isopropanol-water (1:1) fraction of *Ci. reticulata* extract from a HP20SS column, **6** – Isopropanol-water fraction (3:1) of *Ci. reticulata* extract from a HP20SS column, **7** – Isopropanol fraction of *Ci. reticulata* extract from a HP20SS column and **8** – EtOAc wash after isopropanol-water elution of *Ci. reticulata* extract from a HP20SS column; active phytochemicals retained in EtOAc eluent (solid circle); Si gel normal phase TLC eluted with hexanes:EtOAc (1:1);

**(C)** TLC analysis of the *Curcuma longa* crude extract and fractions exposed to elution with various chromatographic media. **1** – Crude *Cu. longa* extract, **2** – Combined *Cu. longa* fractions from a Si gel column, **3** – Isopropanol-water (1:3) fraction of *Cu. longa* extract from a HP20SS column, **4** – Isopropanol-water (1:1) fraction of *Cu. longa* extract from a HP20SS column, **5** – Isopropanol-water (3:1) fraction of *Cu. longa* extract from a HP20SS column, **6** – Isopropanol fraction of *Cu. longa* extract and **7** – EtOAc wash of *Cu. longa* extract from a HP20SS column after isopropanol-water elution; active phytochemicals retained in EtOAc eluent (solid circle); Si gel normal-phased TLC eluted with hexanes:EtOAc (1:1).

Pigments = “bracketed” spots,  $UV_{254}$ -absorbing compounds = “dotted circles” and 10% ethanolic sulfuric acid-charred compounds = “arrows”.



**Figure S2. Inhibition of HIF-1 activation [hypoxia (1% O<sub>2</sub>)-induced and 1,10-phenanthroline (1,10-phen)-induced] by extracts.** Data shown are average + standard deviation ( $n = 3$ ).



**Figure S3. Inhibition of 1,10-phenanthroline-induced HIF-1 activation by recombined fractions obtained by passing *Citrus reticulata* extract over various media.** [1 – Control, 2 – HP20SS, 3 – Si Gel, 4 – Diol, 5 – Sephadex LH-20]. Identical volumes of the stock solutions used to load the columns were placed in vials and dried ( $n = 3$ ). The HIF-1 inhibitory activity of the dried materials served as controls. Data represent average + standard deviation ( $n = 3$ ). Data was analyzed by one-way ANOVA with Bonferroni post hoc test; Differences between data sets were considered statistically significant when  $p < 0.05$  (\*\*\*)  $p < 0.001$ ).



# ANALYSIS REPORT

## ANALYSIS REPORT

Figure S4. HPLC analysis report of *Curcuma longa* powder (McCormick).

**Sample Name:** Nagle/Tumeric (01/2013)

**Assay Method:** UHPLC-UV

**Date of Analysis:** January 24, 2013

**Date of Report:** January 24, 2013

**Compounds Used For UHPLC Chemical Analysis:**

- (1) Curcumin
- (2) Desmethoxycurcumin
- (3) Bisdesmethoxycurcumin
- (4) Ar-turmerone

**Results:**

Sample Name	Sample Weight (mg)	Amount (% , mg/100 mg sample weight)			
		Curcumin	Desmethoxycurcumin	Bisdesmethoxycurcumin	Ar-turmerone
Nagle/Tumeric (01/2013)	15.1	18.8	5.3	7.2	9.5

**Reference:** Bharathi Avula, Yan-Hong Wang and Ikhlas A. Khan, Quantitative Determination of Curcuminoids from the Roots of *Curcuma longa*, *Curcuma* species and Dietary Supplements Using an UPLC-UV-MS Method, *J. Chromatograph. Separat. Techniq.* 2012, 3:120. (doi:10.4172/2157-7064.1000120).

**Analyzed By:** Dr. Yan-Hong Wang

**Reviewed & Approved By:** Dr. Ikhlas A. Khan

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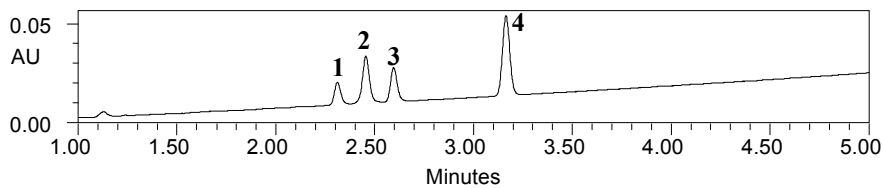
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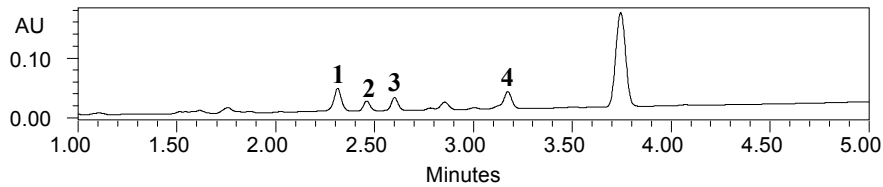
### HPLC Chromatograms

#### UHPLC-UV analysis at 240 nm

**Mixture of Standards [curcumin (1), desmethoxycurcumin (2), bisdesmethoxycurcumin (3) and ar-turmerone (4)]**



#### ***Curcuma longa* (MPG-A104)**



#### **Nagle/Tumeric (01/2013)**

