# Bioactivities of synthesized curcumin, curcuminoids, and their metal complexes

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## Abstract

Curcumin is a compound, derived from the *Curcuma longa* plant, which has shown anticancer activity and relatively low toxicity in humans. It has been a subject of significant interest in recent years as an adjuvant chemotherapy agent. Literature data for curcumin and related curcuminoids have shown discrepancies in their reported bioactivities, and this has spurred disputes in the scientific community about their purported benefits. A possible explanation for these inconsistencies is variability between curcumin sources in purity and content. A systematic study has been conducted on the biological properties of curcumin from various sources, both naturally sourced and synthesized using neat and solvent-based methods. Bioactivities tested included antioxidative capacity and cell growth inhibition. This study has also been expanded to include curcuminoids containing varied electronic and structural features, and biological properties were also evaluated after coordination to a metal.

## Methods

**Antioxidant activity** was determined using the FRAP assay. The FRAP assay was conducted by mixing 10µL 100uM curcuminoid solution in DMSO with 90µL TPTZ reagent (TPTZ, $FeCl_3$-$6H_2O$ in pH 3.6 300mM Acetate buffer). This solution was allowed to incubate for 5 minutes before measuring the absorbance at 593nm. Absorbance at 593nm was determined by a Spectramax M5 instrument in a 96 well plate. These results are reported in Figure 1 aside 50µM ascorbate as a standard.

**UV-Vis spectra** were collected from 300-700nm at concentrations between 75µM and 5µM of curcuminoid and displayed in Figure 2. Absorbances from 300-700nm were similarly determined by a Spectramax M5 in a 96 well plate.

**Antibacterial activity** was determined using the disk diffusion method. Sterile disks were treated with 10µL of 20mg/mL curcuminoid in DMSO and allowed to dry. 150µL of bacterial colonies in the exponential growth phase were placed on Muller Hinton agar plates, then allowed to incubate for 16 hours at 37 ºC with the treated discs. The radius of growth inhibition around the discs in millimeters was determined and reported in Table 1.

## Results

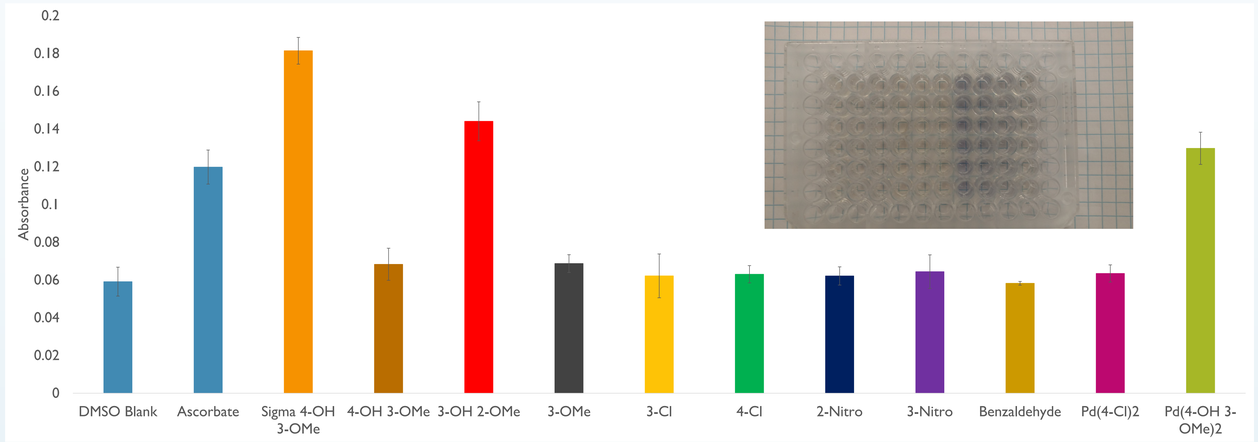



Figure 1. FRAP Values of Curcuminoids. Most curcuminoids remained indistinguishable from the blank in terms of antioxidative activity. Notably, after coordination to a metal, curcumin exhibited decreased antioxidant activity. Bars are the result of nine trials and error bars are determined by the standard deviation of these trials. Concentration of curcuminoid was 10µM/mL. Inset shows an example of the color change observed in the FRAP assay. Though some curcuminoids demonstrated significant absorbance at 593nm, (see Figure 2) this should not have affected the FRAP assay results.

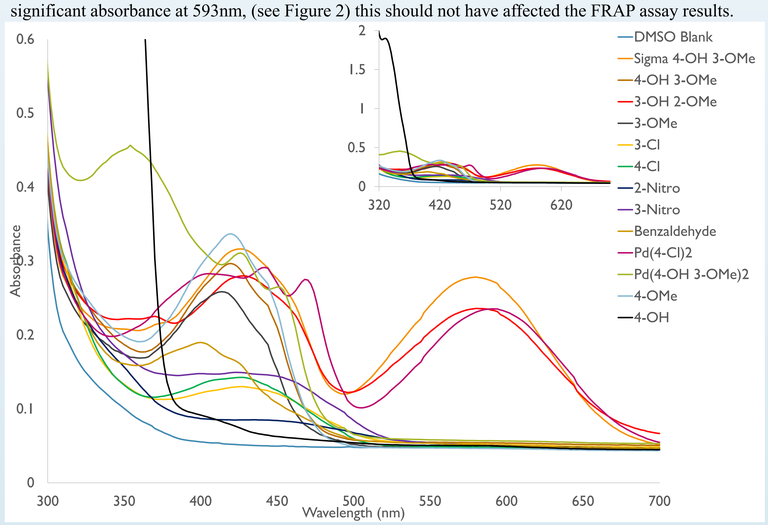



Figure 2. UV-Vis Spectra of Curcuminoids. Most curcuminoids demonstrated UV-Vis spectra similar to curcumin itself, with absorbance maxima between 400 and 450nm. Concentrations in the UV-Vis spectra were as follows. 75uM: 3-Cl, 2-Nitro, 3-Nitro, Benzaldehyde. 30uM: 4-OH, 3OMe, Sigma 4-OH, 3OMe, 3-OH, 2OMe, 3-OMe, 4-Cl, Pd(4-OH, 3-OMe)2, 4OMe. 15uM: Pd2Cl2, Pd-4Cl. 5uM: 4-OH. Some curcuminoids demonstrated significant absorbance at 593nm, which is used for the FRAP assay determination. Inset shows large absorbance between 320nm and 400nm of 4-OH curcuminoid.

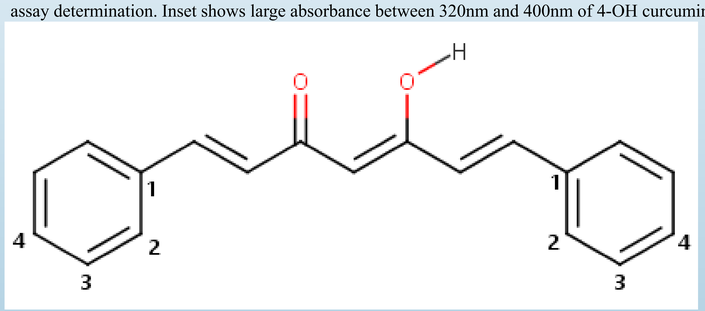
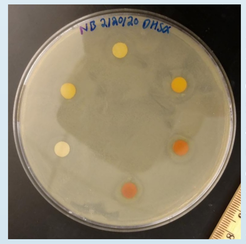
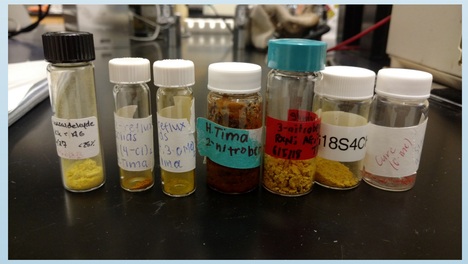



Figure 3. General Structure of Curcuminoids. Curcuminoids used in this research possess the same general structure shown above, but vary in placement of functional groups on the aromatic rings.

| Species, zone of inhibition (mm) | *E. Coli* DH5α | *B. Subtillis* | *S. aureus* | *P. aeruginosa* |
|---|---|---|---|---|
| DMSO Blank | 11 | no effect | 7 | no effect |
| Sigma 4-OH 3-OMe | 11 | no effect | no effect | no effect |
| 4-OH 3-OMe | 10 | no effect | no effect | no effect |
| 3-OH 2-OMe | 13 | no effect | no effect | no effect |
| 3-Cl | 12 | 7 | 7 | 7 |
| Pd(4-OH 3-OMe)2 | 12 | 7 | 7 | 7 |

Table 1. Disk Diffusion Assay. Curcuminoids appeared most effective against *E. Coli* DH5α, however this activity was not significantly different from the blank.





Figures 4 and 5. Examples of Disk Diffusion Assay and of Curcuminoids.
Shown to the left is an example of a disk diffusion assay performed using DH5α. Shown below is an example of the curcuminoids used in this research. Most curcuminoids were yellow in color as solids and in solution.

## Conclusion

Most curcuminoids demonstrated antioxidant activity indistinguishable from the blank, supporting the conclusion from previous research that antioxidant activity is tied to the hydroxyl group on the aromatic rings. Additionally, coordination to a metal decreased curcumins antioxidant activity. Curcuminoids demonstrated little to no antibacterial activity against bacteria used in the disk diffusion assay. Against DH5α, some amount of antibacterial activity was demonstrated, however this activity was not significantly different from the blank. Poor solubility in the agar medium used for bacterial growth could explain why no significant antibacterial activity was measured.

In the future, it would be beneficial to determine the antioxidant capabilities of curcuminoids which maintain the alcohol group while varying electron withdrawing and donating groups around the aromatic rings. Additionally, using the MIC assay to determine antibacterial activity could be a more reproducible and comparable method of determining antibacterial activity.

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