

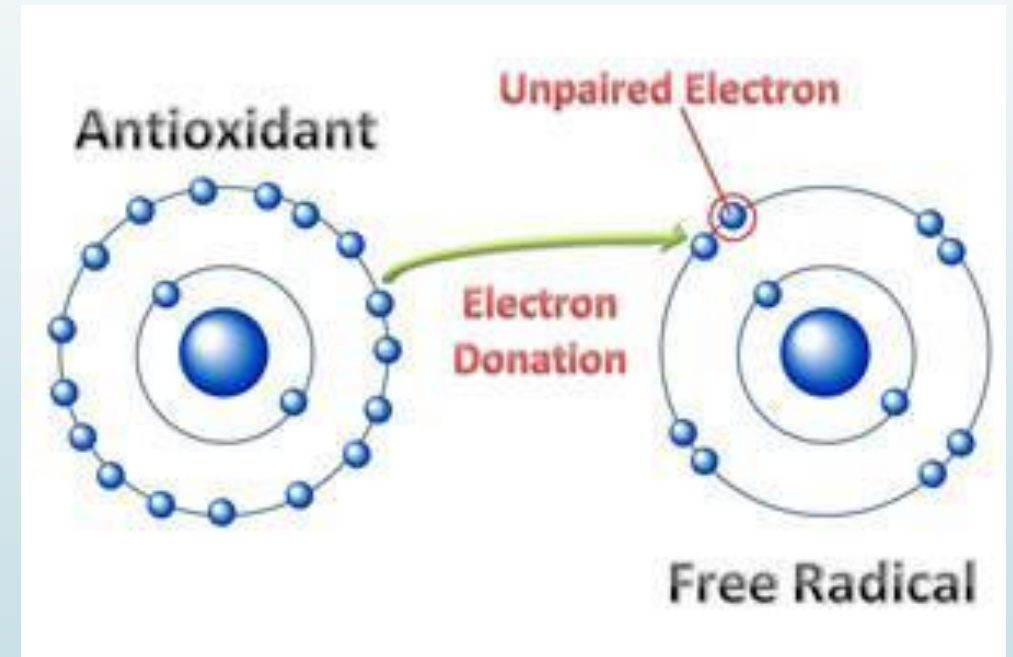


# Determining the Antioxidant Capacity and Antimutagenicity/Mutagenicity of Quercetin and Ginger

Caitlin Castillo

# Free Radicals and Antioxidants

- ▶ Free Radicals are unstable molecules with unpaired electrons
- ▶ Reactive oxidant species (ROS) and free radicals can increase the risk of cancer and cardiovascular diseases
- ▶ Antioxidants are known to reduce oxidative stress
- ▶ They are studied to determine their effect against ROS and free radicals





# Antioxidants and Cancer

- Antioxidants have been suspected to decrease risk of cancer
- There were mixed results on whether antioxidant supplements prevented cancer in some clinical trials
- Some people already diagnosed with cancer had worse outcomes and had progression of tumor growth
- Antioxidants protect healthy cells and cancer cells from oxidative stress
- When paired with chemotherapy drugs, increase therapeutic response

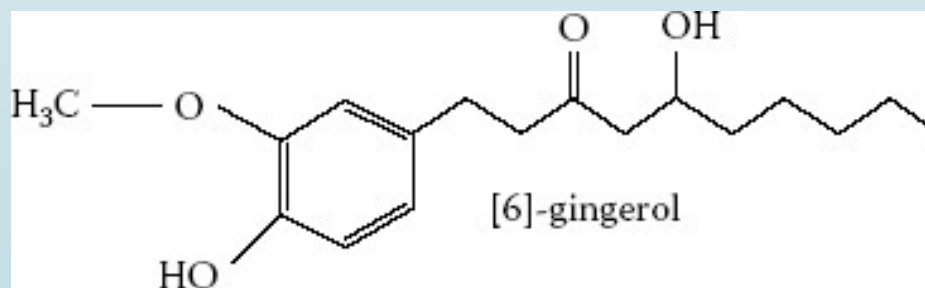
# Objectives

- ▶ Determine antioxidant capacity in quercetin and ginger
- ▶ Decide if quercetin or ginger are antimutagenic
- ▶ Establish the effective concentration



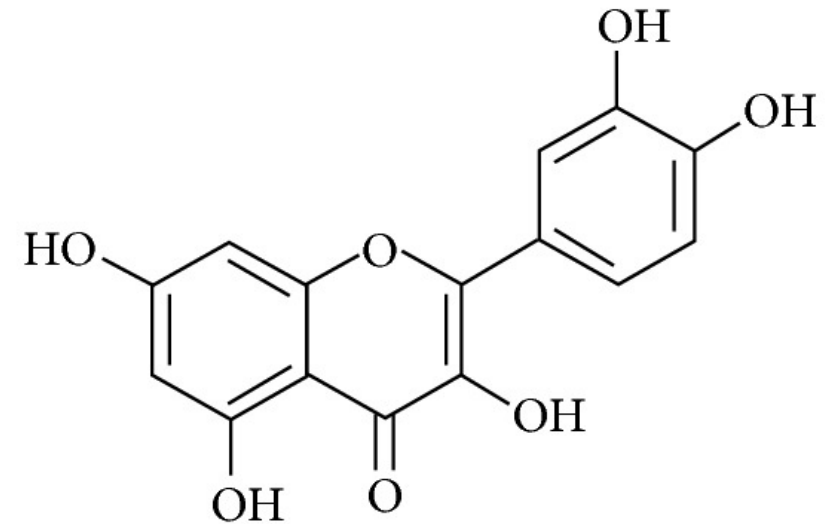
# Ginger

- Ginger root is generally used as a spice in many foods and beverages
- Known to be high in antioxidants
- Decreases oxidative stress in human leukemia cells
- Active ingredient is 6-gingerol



# Quercetin

- ▶ A plant flavanol found in many foods
- ▶ Can be taken as a supplement
- ▶ Has antioxidant, anti-inflammatory, and anticarcinogenic properties
- ▶ Contradictory *in vitro* vs. *in vivo* results
- ▶ Helped prevent Squamous Cell Carcinoma (SCC) in mice



# Phosphomolybdate Assay

0.1 mL  
antioxidant  
sample



1 mL reagent  
( $\text{H}_2\text{SO}_4$ ,  $\text{Na}_3\text{PO}_4$ ,  
ammonium  
molybdate)



Incubate at  $95^\circ\text{C}$  for  
30 minutes

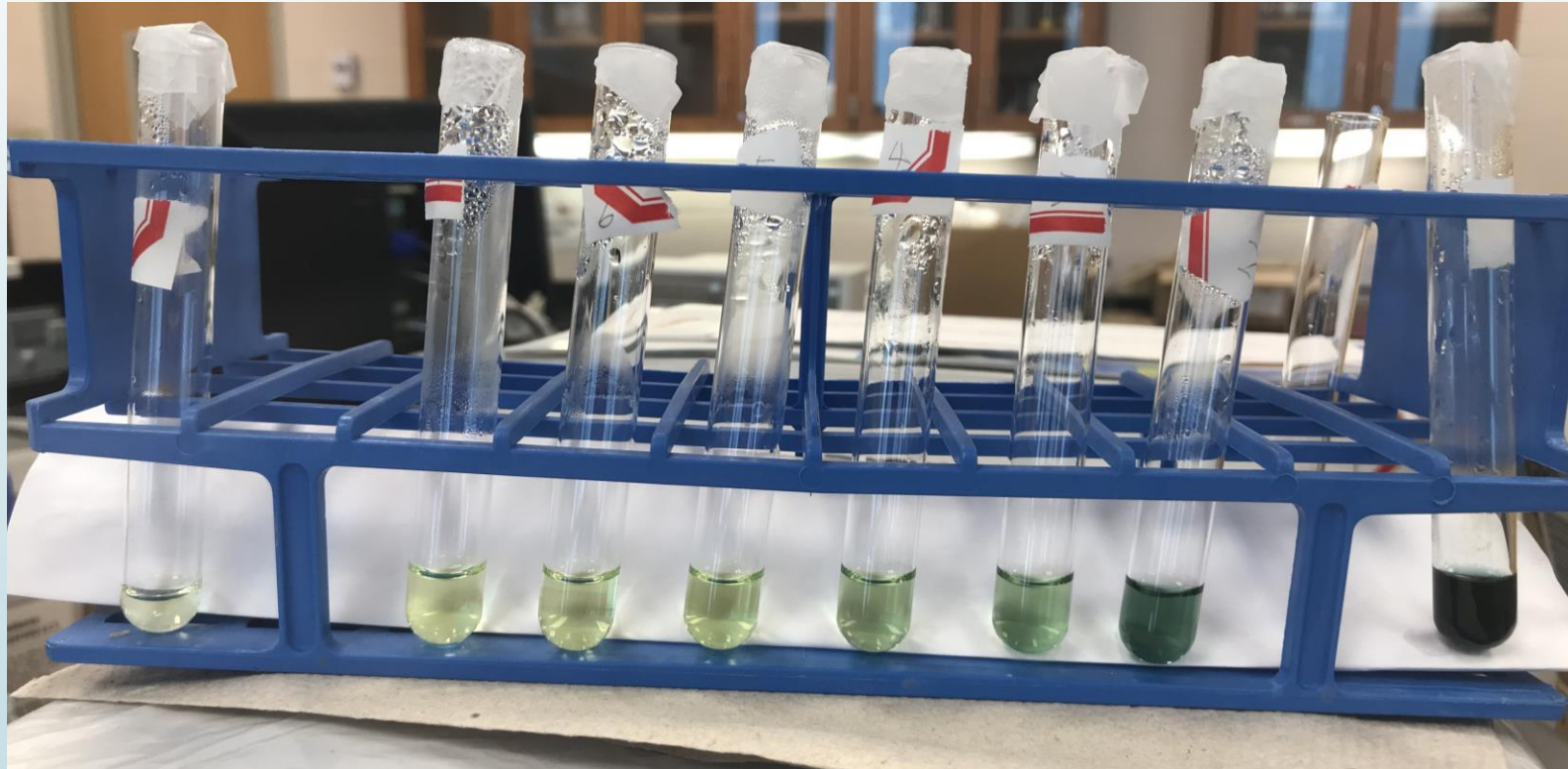
Allow samples to cool then  
measure absorbance at 695 nm



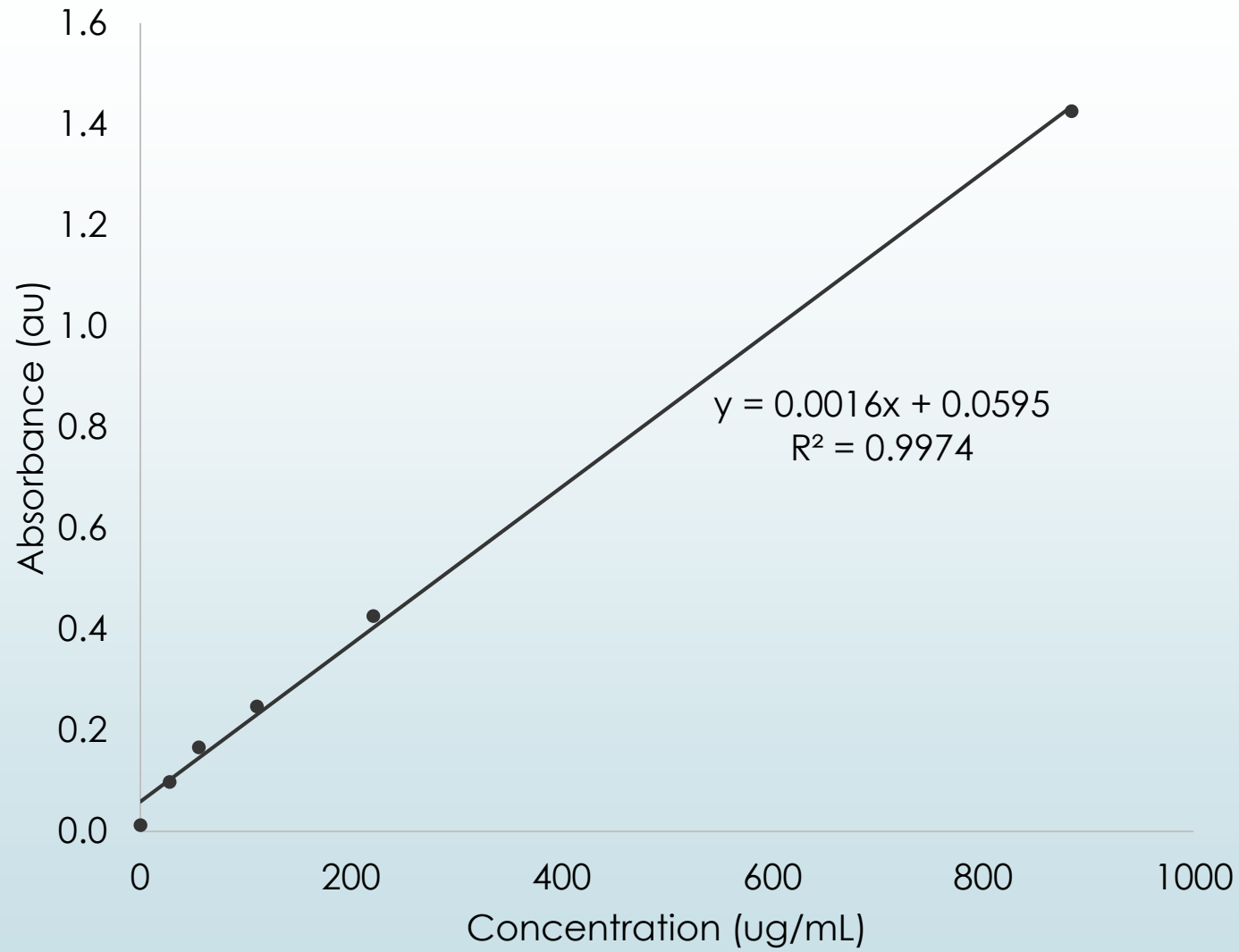


# Phosphomolybdate Assay with Quercetin

Blank	27.5	55	110	220	440	880	3520
	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL







Phosphomolybdate  
Assay with  
Quercetin



# Using the Ames Test to Determine Mutagenicity of a Substance

- **Mutagen**- an environmental or chemical agent that causes a genetic mutation
- **Salmonella typhimurium**- type of bacteria used in Ames Test
- **Strain TA1535**- has a missense mutation from cytosine to guanine changing the amino acid from leucine to proline, making the enzyme for histidine synthesis inactive
- **Strain TA1538**- has a deletion of a base pair that causes a frameshift and early stop codon, making the enzyme for histidine inactive
- **Revertant**- organism or strain that has mutated back to the wild type from mutant type

# Ames Test

- Developed in the 1970s to determine the mutagenicity of a test substance
- If a substance was mutagenic, then can be considered carcinogenic
- Reversion frequency determines mutagenicity of a substance
- Antioxidants have antimutagenic effects and properties
- Predicting that Quercetin and Ginger will be antimutagenic

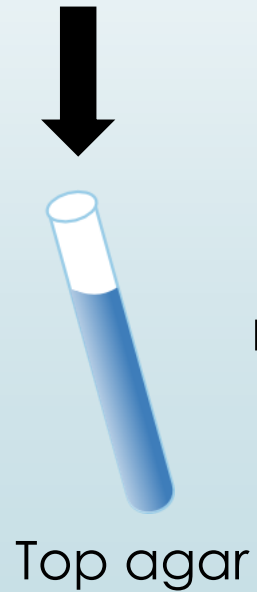


# Reversion Frequency

- $RF = \frac{\text{\# of revertant colonies in minimal his media/mL}}{\text{\# of colonies in rich his media/mL}}$
- RF 2x the negative control = mutagenic
- RF < negative control = antimutagenic

# Ames Test Protocol

1. Quercetin/Ginger
2. TA1535 or TA1538



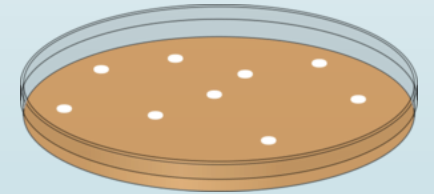
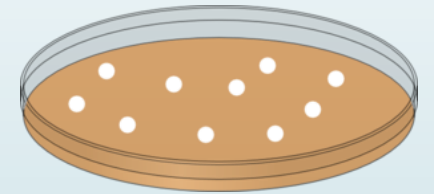
VBM plate  
(minimal  
histidine)



Add  
mutagen



Incubate for  
2 days at  
37°C



Count colonies

	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6
<i>Salmonella</i> strain	TA1535	TA1538	TA1535	TA1538	TA1535	TA1538
Test Substance	ETOH	ETOH	None	None	Ginger	Ginger
Mutagen?	No	No	NaN3	4-NOP	NaN3	4-NOP
	Plate 7	Plate 8	Plate 9	Plate 10	Plate 11	Plate 12
<i>Salmonella</i> strain	TA1535	TA1538	TA1535	TA1538	TA1535	TA1538
Test Substance	Ginger + H2O	Ginger + H2O	Quercetin	Quercetin	Quercetin	Quercetin
Mutagen?	No	No	No	No	NaN3	4-NOP

4-NOP = 4-nitro-O-phenylenediamine



# Quercetin and the Ames Test

- Concentrations to be tested: 45 ug/mL, 75 ug/mL, 220 ug/mL, 880 ug/mL and 3520 ug/mL
- Quercetin is given in extremely high doses/concentrations to help prevent SCC and has known anticancer effects *in vivo*





# Ginger and the Ames Test

- ▶ Ginger was heat treated to be sterilized
- ▶ Extract was used for the Ames Test
- ▶ Prior data showed that urinary-mutagen exposed rats being fed a ginger diet had antimutagenic effects

# Results

TA1535	TA1535	TA 1535	TA 1535	TA 1535
Positive control	Negative control	Ginger + NaAz	150 uM Quercetin + NaAz	250 mM Quercetin + NaAz
2.97E-07	2.55E-08	4.40E-07	1.10E-07	4.26E-08

TA1538	TA1538	TA1538	TA1538	TA1538
Positive control	Negative control	Ginger + 4-NOP	150 uM Quercetin + 4-NOP	250 mM Quercetin + 4-NOP
4.84E-07	6.36E-07	7.98E-08	5.35E-06	-

TA 1535	TA 1535	TA 1535	TA 1538	TA 1538	TA 1538
Ginger + H2O	150 uM Quercetin + H2O	250 uM Quercetin + H2O	Ginger + H2O	150 uM Quercetin + H2O	250 uM Quercetin + H2O
6.00E-09	1.20E-08	3.30E-08	6.30E-08	3.90E-08	3.80E-07



## Future work

- ▶ Test cooked vs. uncooked ginger using phosphomolybdate assay
- ▶ Complete Ames test using varying concentrations of quercetin
- ▶ Analyze data and determine mutagenicity/antimutagenicity of ginger and quercetin

# Thank you

- ▶ Xavier University Chemistry Department
- ▶ Dr. Supaporn Kradtap
- ▶ Jacob Demott
- ▶ Mr. Steve Nichols
- ▶ Dr. Dorothy Engle, Biology Department
- ▶ Taylor Chae and the Genetics Students
- ▶ Davies Lab at Cincinnati Children's Hospital





Questions?