**Introduction**

**Free Radicals:**
- Chemicals with a lone unpaired electron
- Linked to health issues from aging to cancer
- Successful antioxidant reacts with unpaired electron and neutralizes the radical

The DPPH Method of Determining Antioxidant Strength
- 2,2-diphenyl-1-picrylhydrazyl (DPPH) exists as a purple solution in the stable radical form
- DPPH exists as a yellow solution when neutralized by an antioxidant
- Spectrophotometer measures change in absorbance at 515 nm to determine how much radical has been neutralized

**Abstract**

Using colored DPPH radical solution various suspected antioxidants were tested and ranked according to their ability to neutralize the DPPH radical.

**Procedure**

**Create DPPH Solution of 1mM in Methanol**

**Create Sample Solution of 500 µg/mL**

**Prepare Wells with Methanol Blank, Initial DPPH Solution, and Mixture of DPPH and Sample**

**Allow 30 Minutes for Solutions to React in Dark and Analyze With Spectrophotometer at 515 nm**

**Determine if Activity Exists**

**Disregard Weak Sample**

**Prepare Sample Dilutions**

**Run Sample Dilutions with DPPH Mixture Using the Spectrophotometer as Before**

**Determine Sample EC50 Value**

**Determining %DPPH Remaining**

\[ \text{Abs(solution) - AbsBlank} \times 100\% = \%\text{DPPH Remaining} \]

The stronger antioxidant produces a smaller %DPPH remaining value

**Determining EC50**

The concentration required to neutralize 50% of the DPPH (EC50) was found by plotting the natural logarithm graph of %DPPH remaining versus the concentration of sample. If sample could not neutralize 50% of the DPPH with concentration 500 µg/mL, then it was discarded as a weak antioxidant.

**Results**

**Conclusions and Future Work**

From the 40+ samples evaluated under this experiment at least ten were found to be potentially powerful antioxidants. The plants from which these extracts were obtained should be further analyzed to maximize the possibility of using these in food, drug, and other medical uses.

Future assays on the strong antioxidants will be used to verify the results of this method and perhaps redeem some of the samples that did not exhibit neutralization ability. These assays include the Ferric Reducing Ability of Plasma (FRAP), Nile Blue, and Dimethylthiazole (MTT) methods.

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