Antioxidant Assay: The DPPH Method
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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 515nm to determine how much radical has been neutralized

The stronger antioxidant produces a smaller %DPPH remaining value

Reactions of DPPH with Antioxidant (RH) and Radical (R)

Abstract

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

Procedure

1. Create DPPH Solution of 1mM in Methanol
2. Create Sample Solution of 500 µg/mL
3. Prepare Wells with Methanol Blank, Initial DPPH Solution, and Mixture of DPPH and Sample
4. Allow 30 Minutes for Solutions to React in Dark and Analyze With Spectrophotometer at 515nm
5. Run Sample Dilutions with DPPH Mixture Using the Spectrophotometer as Before
6. Determine if Activity Exists
7. Disregard Weak Sample
8. Prepare Sample Dilutions
9. Determine Sample EC50 Value

DPPH Method of Determining Antioxidant Strength

DPPH exists as a purple solution when neutralized by an antioxidant
Successful antioxidant reacts with unpaired electron and neutralizes the radical
Spectrophotometer measures change in absorbance at 515nm to determine how much radical has been neutralized

Determining %DPPH Remaining

AbsSolution - AbsBlank \times 100\% = %DPPH Remaining

AbsSolution - AbsBlank = %DPPH remaining value

Reactions of DPPH with Antioxidant (RH) and Radical (R)

Results

Dilutions of Antioxidants

Absorbance at 515nm vs. Concentration DPPH

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 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.

Acknowledgements

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- Dr. Setzer and Dr. Vogler for the usage of the Natural Products Lab Facilities and for their continued support

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC50 Value (µg/mL)</th>
<th>Standard Deviation</th>
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<tbody>
<tr>
<td>Catechin</td>
<td>19.99</td>
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<td>Tannic Acid</td>
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<td>Trolox</td>
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