Antioxidant Assay: The DPPH Method

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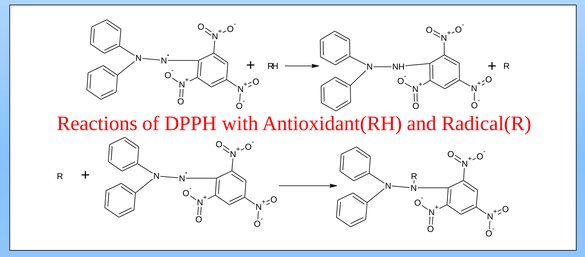
Introduction

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

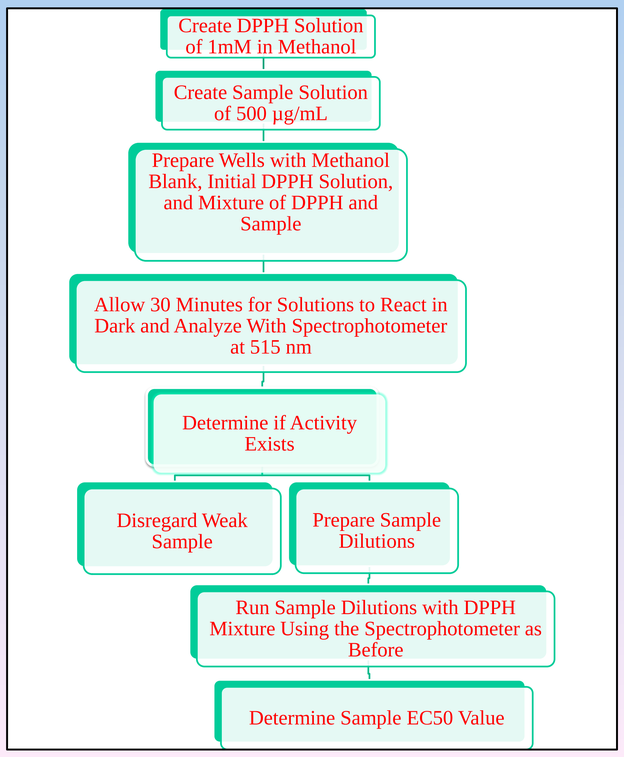


Reactions of DPPH with Antioxidant(RH) and Radical(R)

Abstract

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

Procedure



Well plate with 1mM DPPH dilutions and reaction of .5mM with Tannic Acid and Trolox Respectively

Determining %DPPH Remaining

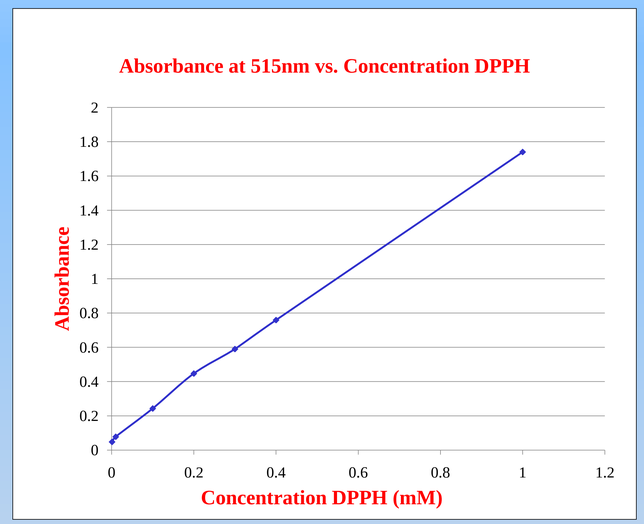
$$\frac{Abs_{solution} - Abs_{blank}}{Abs_{initial} - Abs_{blank}} \times 100\% = \%DPPH\ Remaining$$

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 500µg/mL, then it was discarded as a weak antioxidant.

Results



Samples With Strong Antioxidant Properties (EC50 Values Below 500)

Sample	EC50 Value (µg/mL)	Standard Deviation
Catechin	19.99	1.57
Tannic Acid	21.78	1.07
Trolox	36.44	0.78
PACARM '96	64.17	3.10
OCFLBM '04	109.52	12.75
INSIBM'07	153.75	1.39
EXBABM '05	163.35	4.03
COXABM '05	203.60	8.20
MUURBM'07	225.04	5.80
LOMOBA-6	276.08	4.15
Hesperetin	331.75	17.15
LOMOBA	360.06	7.46
MAUELM '05	394.33	6.33
BERBLM '05	402.73	5.12

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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