The Folin-Ciocalteu Assay as a Valid Means of Assessing Antioxidant Activity of Compounds of Biomedical Interest

By:

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Background

- The Folin-Ciocalteu (FC) Assay was developed in 1927 as a colorimetric assay for tyrosine.
- The FC assay uses a mixture containing sodium molybdate, sodium tungstate, and other reagents.
- In the presence of phenols, it produces a blue color which absorbs at 765 nm. It is believed that the blue color is due to a complexed Mo(V) species.
- The FC assay is used to measure phenolic content in plants.
- A modification of it, called the Folin-Lowry assay is used to measure total protein content of fluids.

Question

- Is the FC assay only useful in measuring phenols and proteins, or does it possibly have other applications?

Observations on Reactivity of Folin Reagent

- Folin and Ciocalteu noted in 1927 noted that the reagent was reactive towards Trp as well as Tyr. This seems to imply that the reactivity of FC reagent is not limited to phenols.
- Numerous investigators over the years reported apparent reactivity of FC reagent with nonphenolic substances
- Ikawa and co-workers in 2003 measured the reactivity of FC reagent towards dozens of nitrogenous compounds. Many had significant reactivity.
- The reactivity of other compound classes towards the FC reagent has not yet been explored

Implications of Observations

- The FC assay may not give an accurate estimation of phenolic contents of plants which also contain other FC-reactive substances
- It is now believed that the FC assay functions as a nonspecific antioxidant assay.
- If this is proven correct, the FC assay may prove useful for measuring the antioxidant capacities of compounds of biomedical interest.

Purpose of Study

- The purpose of our study is to determine the reactivity of the FC reagent towards different classes of compounds. The classes examined include:

- 1) Thiols 2) Vitamins 3) Proteins 4) Amino acids 5) Nucleotide bases 6) Unsaturated fatty acids 7) Carbohydrates 8) Organic acids 9) Aldehydes and ketones 10) Inorganic ions

Activity of Phenolic Compounds

Compound	Formula weight	GAE(mass)	GAE (molar)
Caffeic acid	180.16	1.002	0.958
Chlorogenic acid	354.31	0.722	1.362
Curcumin	368.4	0.722	1.411
Ellagic acid	302.19	1.321	2.122
Ferulic acid	194.18	1.050	1.083
Gallic acid	188.14	1.000	1.000
Quercetin	338.26	1.160	2.080
Resveratrol	228.25	1.013	1.230
Rutin	610.52	0.568	1.521
Salicylic acid	138.12	0.357	0.262
Tannic acid	~1700	0.878	9.040

Activity of Thiol Derivatives

Compound	Formula weight	GAE (mass)	GAE (molar)
Amifostine	214.22	0.378	0.430
Captopril	217.29	0.323	0.373
Cysteamine	113.61	0.304	0.184
Glutathione	307.3	0.161	0.263
MPG	163.2	0.342	0.297
Penicillamine	149.21	0.333	0.264
PTCA	175.25	0.180	0.141
RibCys	253.23	0.202	0.268
WR-1065	134.24	0.375	0.268
N-acetylcysteine	163.2	0.395	0.342
Zn-NAC	228.59	0.187	0.227
Zn-RibCys	318.62	0.272	0.460

Activity of Vitamin Derivatives

Compound	Formula weight	GAE(mass)	GAE (molar)
Ascorbic acid	176.12	0.662	0.664
Biotin	244.31	–	–
Folic acid	441.40	0.071	0.167
Folinic acid	473.44	0.069	0.174
Menadione	172.18	–	–
NADH	709.4	.0054	0.204
Nicotinic acid	123.11	–	–
Pyridoxine	205.64	0.211	0.231
Retinoic acid	300.42	0.404	0.645
Riboflavin	376.36	–	–
Thiamine	337.28	0.183	0.328
Trolox	250.29	0.395	0.525

Reactivity of Amino Acids

Compound	Formula weight	GAE (mass)	GAE (molar)
Alanine	89.09	–	–
Arginine	174.20	–	–
Cysteine	121.16	0.281	0.181
Glycine	75.07	–	–
Histidine	155.16	–	–
Hydroxyproline	131.13	–	–
Lysine	146.19	–	–
Methionine	149.21	–	–
Tryptophan	204.22	0.413	0.448
Tyrosine	181.89	0.397	0.382

Reactivity of Nucleotide Bases and Unsaturated Fatty Acids

Compound	Formula weight	GAE (mass)	GAE (molar)
Adenine	135.13	0.022	0.016
Cytosine	111.10	–	–
Guanine	151.13	0.340	0.273
Thymine	126.11	–	–
Arachidonic acid	304.5	0.005	0.016
Linolenic acid	280.45	–	–
Linoleic acid	278.42	–	–

Reactivity of Carbohydrates and Proteins

Compound	Formula weight	GAE (mass)	GAE (molar)
Dihydroxyacetone	90.08	0.044	0.021
D-deoxyribose	164.16	–	–
D-glucose	180.16	–	–
D-glyceraldehyde	90.08	0.024	0.012
D-lactose	342.3	–	–
D-maltose	342.3	–	–
Methylcellulose	~ 440 kd	–	–
Potato starch	~ 1000 kd	–	–
D-sucrose	342.3	–	–
Egg Albumin	~ 65 kd	0.016	5.630
Bovine Albumin	69.3 kd	0.028	10.390

Reactivity of Aldehydes, Ketones and Carboxylic Acids

Compound	Formula weight	GAE (mass)	GAE (molar)
Cinnamaldehyde	132.16	–	–
Citronellal	154.25	–	–
Alpha ionone	192.3	0.004	.004
2,3-Butanedione	86.09	0.018	.008
Cinnamic acid	148.17	–	–
Citric acid	192.12	–	–
Oxalic acid	126.07	–	–
Quinic acid	197.17	–	–
Tartaric acid	174.07	–	–

Reactivity of Inorganic Salts and Other Compounds

Compound	Formula weight	GAE (mass)	GAE (molar)
Iron (II) chloride	126.73	0.149	0.100
Mn (II) chloride	125.84	0.043	0.029
Sodium nitrite	85.01	–	–
Sodium sulfite	126.04	0.051	0.034
Potassium iodide	166.00	0.022	0.020
Caffeine	194.19	–	–
Cystamine	225.20	–	–
GSSG	612.60	–	–
Menthol	156.27	–	–

Abbreviations Used in Next Slides

- DIPS = 2,5-Diisopropylsalicylic acid
- DBS = 2,5-Dibromosalicylic acid
- DTBS = 2,5-Di-tertiary butyl salicylic acid
- DCS = 2,5-Dichlorosalicylic acid
- These compounds are of interest because their Zn(II), Mn(II) and Cu(II) complexes have shown very desirable cytoprotective properties.

Reactivity of Substituted Salicylic Acids and their Metal Complexes

Compound	Formula weight	GAE (mass)	GAE (molar)
Salicylic acid	138.12	0.357	0.262
DIPS	222.28	0.241	0.285
DBS	295.93	0.135	0.213
DTBS	250.34	0.055	0.074
Zn-DIPS	543.92	0.088	0.253
Cu-DIPS	1084.25	0.071	0.411
Cu-DBS	1378.78	0.088	0.647
Zn-DTBS	600.08	0.033	0.106
Cu-DTBS	1196.47	0.020	0.126
Cu-DCS	1023.17	0.077	0.438

Salicylate Derivatives Results

- All ligands and complexes tested had significant reactivity towards the FC reagent.
- The di-tertiary butyl derivatives had the least activity.
- Metal complexation, especially copper (II) appeared to increase reactivity slightly.

Comparison of FC and ABTS Assays

Compound	Trolox Equivalents (FC)	Trolox Equivalents (ABTS)
Gallic acid	1.90	2.60
Quercetin	3.96	4.95
Caffeic Acid	1.82	1.76
Rutin	2.89	3.30
Chlorogenic Acid	2.58	2.00
Curcumin	2.68	2.89
Amifostine	0.87	1.60
Captopril	0.71	0.90
Cysteamine	0.35	0.40
N-Acetylcysteine	0.65	0.88
Penicillamine	0.50	1.54

FC vs ABTS

- Reactivity of phenolic compounds between the two assays correlates quite well. P = 0.9
- There is a moderate correlation seen among the thiol derivatives. P = 0.6 Amifostine and penicillamine seem to have special reactivity towards ABTS which is not reflected in their reactivity towards the FC reagent.

OVERALL SUMMARY

- Our study showed that reactivity of the Folin – Ciocalteu reagent is not limited to phenols.
- Many other compounds of varying classes also show reactivity towards the reagent.
- Reactivity towards the reagent should be seen as a measure of overall antioxidant capacity rather than phenolic content.
- The assay could be used as an antioxidant assay to assess the total antioxidant capacities of compounds of biomedical interest.

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