

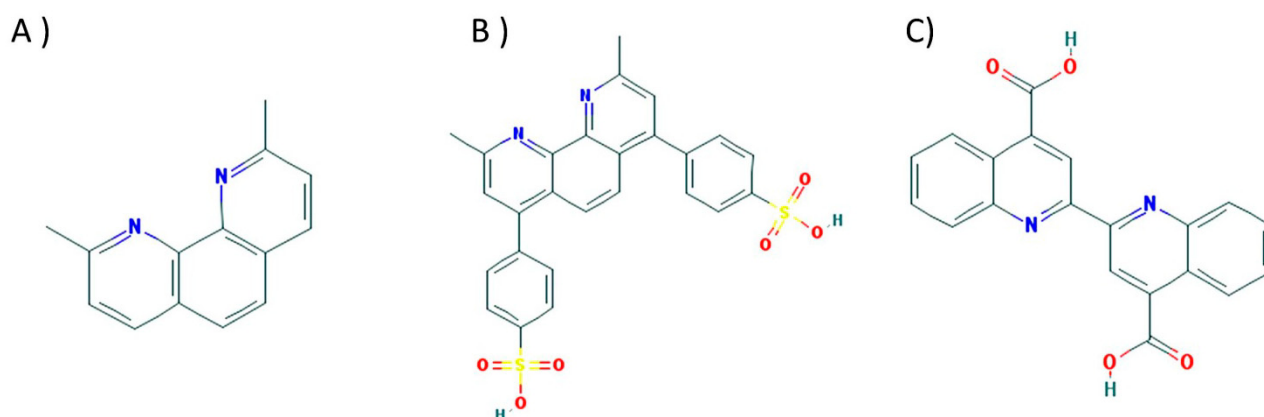
## Supplementary Information

**Table S1.** Summarized experimental conditions for the studied copper reducing antioxidant assays.

| Reagent          | Volume      | Neocuproine (NC) <sup>a</sup> | Bathocuproine (BCS) <sup>a</sup> | Bicquionic Acid (BCA) <sup>a</sup> |
|------------------|-------------|-------------------------------|----------------------------------|------------------------------------|
| Cu <sup>2+</sup> | 50 $\mu$ L  | 10 mM                         | 10 mM                            | 2.0 mM                             |
| Reagent          | 50 $\mu$ L  | 7.5 mM                        | 7.5 mM                           | 6.0 mM                             |
| Buffer           | 50 $\mu$ L  | 1.0 M                         | 1.0 M                            | 1.0 M                              |
| AOX              | 100 $\mu$ L |                               | 0–0.1 mM                         |                                    |
| Wavelength       |             | 450 nm                        | 485 nm                           | 558 nm                             |
| Assay time       |             |                               | 1 h                              |                                    |
| Assay pH         |             |                               | 7.0                              |                                    |

<sup>a</sup> Concentration values prior to addition to micro plate wells.

**Figure S1.** Structure of applied complexing agents, (A) Neocuproine (2,9-dimethyl-1,10-phenanthroline) NC; (B) Bathocuproine disulfonic acid (2,9-dimethyl-4,7-diphenyl-1,10-phenantrolinedisulfonic acid) BCS; (C) Bicinchoninic acid (2-(4-carboxyquinolin-2-yl)quinoline-4-carboxylic acid) BCA. The chemical structure information for these molecules is available in the PubChem Substance and Compound database through the unique chemical structure identifier NC-CID 65237; BCS-CID 170300 and BCA-CID 71068.



**Table S2.** Calibration curves obtained using the BCS complex for the studied antioxidant compounds.

| Antioxidant   | Intercept $\pm$ Standard Error <sup>a</sup> | Slope $\pm$ Standard Error <sup>a</sup> , $\mu\text{M}^{-1}$ | $R^2$  |
|---------------|---------------------------------------------|--------------------------------------------------------------|--------|
| Trolox        | $0.103 \pm 0.002$                           | $(6.50 \pm 0.04) \times 10^{-5}$                             | 0.9998 |
| Ascorbic acid | $0.101 \pm 0.002$                           | $(7.44 \pm 0.03) \times 10^{-5}$                             | 0.9999 |
| Uric acid     | $0.103 \pm 0.002$                           | $(12.24 \pm 0.04) \times 10^{-5}$                            | 0.9999 |
| Glutathione   | $0.118 \pm 0.006$                           | $(4.1 \pm 0.1) \times 10^{-5}$                               | 0.9960 |

The estimated overall regression parameters correspond to the results obtained in separate working days (5 working concentrations,  $n = 4$ ). <sup>a</sup> Estimate of the linear regression parameters (for 5 freshly prepared standard solutions in the range of 0 to 100  $\mu\text{M}$  analyzed in quadruplicate).

**Table S3.** Equation parameters, covariance data ( $TE_{det.} = a + b \times TE_{calc.}$ ) for assays containing binary mixtures of antioxidants.

| Composition <sup>a</sup>  | a <sup>b</sup>     | b <sup>b</sup>  | R      |
|---------------------------|--------------------|-----------------|--------|
| Trolox/ascorbic acid      | $0.002 \pm 0.001$  | $0.96 \pm 0.04$ | 0.9983 |
| Trolox/uric acid          | $-0.001 \pm 0.001$ | $1.00 \pm 0.03$ | 0.9991 |
| Trolox/glutathione        | $0.002 \pm 0.002$  | $0.99 \pm 0.04$ | 0.9981 |
| ascorbic acid/uric acid   | $-0.004 \pm 0.002$ | $1.02 \pm 0.02$ | 0.9996 |
| ascorbic acid/glutathione | $0.001 \pm 0.002$  | $1.10 \pm 0.04$ | 0.9990 |
| uric acid/glutathione     | $0.002 \pm 0.002$  | $1.04 \pm 0.03$ | 0.9994 |

<sup>a</sup>  $n = 12$  different solutions (Table S6) containing the two antioxidants in different ratios were analysed in quadruplicate; <sup>b</sup> value  $\pm$  confidence interval ( $\alpha = 0.05$ ).

**Table S4.** Intra-, Inter assay precision for ascorbic acid, uric acid, glutathione and Trolox.

| Conc. ( $\mu$ M) | Ascorbic Acid |     | Uric Acid |     | Glutathione |     | Trolox |     |
|------------------|---------------|-----|-----------|-----|-------------|-----|--------|-----|
|                  | WD%           | BD% | WD%       | BD% | WD%         | BD% | WD%    | BD% |
| 10               | 1.2           | 7.0 | 0.6       | 5.7 | 1.7         | 4.0 | 1.1    | 5.3 |
| 20               | 0.5           | 4.2 | 0.7       | 3.5 | 1.2         | 2.9 | 1.1    | 3.5 |
| 40               | 1.1           | 4.7 | 0.4       | 4.3 | 1.1         | 1.8 | 0.9    | 1.5 |
| 60               | 0.8           | 4.5 | 0.7       | 3.0 | 1.2         | 1.8 | 0.8    | 1.7 |
| 100              | 1.3           | 2.4 | 0.4       | 1.5 | 0.9         | 1.7 | 0.6    | 0.9 |

WD% intra (within day) precision, BD% inter (between day) precision,  $n = 4$ ,  $d = 3$ .

**Table S5.** Stability of sample assessed under different storage conditions.

| Sample                | Initial | 2 Weeks at $-18^\circ\text{C}$ | 3 Freeze/Thaw Cycles | 24 h Room Temperature |
|-----------------------|---------|--------------------------------|----------------------|-----------------------|
| Average ( $\mu$ M TE) | 28.9    | 27.7                           | 27.6                 | 25.6                  |
| SD                    | 0.5     | 0.4                            | 0.4                  | 0.4                   |
| Variation             | -       | -4.1%                          | -4.5%                | -11.4%                |

**Table S6.** Preparation of binary mixtures of antioxidants.

| Assay No. | [AOX <sub>1</sub> ]/ $\mu$ M | [AOX <sub>2</sub> ]/ $\mu$ M | Ratio of [AOX <sub>1</sub> ]/[AOX <sub>2</sub> ] |
|-----------|------------------------------|------------------------------|--------------------------------------------------|
| 1         | 10                           | 10                           | 1                                                |
| 2         | 20                           | 10                           | 2                                                |
| 3         | 30                           | 10                           | 3                                                |
| 4         | 40                           | 10                           | 4                                                |
| 5         | 50                           | 10                           | 5                                                |
| 6         | 10                           | 20                           | 0.5                                              |
| 7         | 10                           | 30                           | 0.3                                              |
| 8         | 10                           | 40                           | 0.25                                             |
| 9         | 10                           | 50                           | 0.2                                              |
| 10        | 20                           | 20                           | 1                                                |
| 11        | 30                           | 30                           | 1                                                |
| 12        | 40                           | 40                           | 1                                                |

Solutions were prepared by dilution of 0.2 mM antioxidant solutions in a final volume of 1.0 mL.