10.

Antioxidant Activity

Anti-oxidants are substances that when present in low concentrations, compared to an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Oxidation of the substrate is initiated by a pro-oxidant, which is a toxic substance, may initiate this oxidation, and cause damage to lipids, proteins and nucleic acids. Pro-oxidants include reactive oxygen and nitrogen species (ROS and RNS). ROS include superoxide ($O_2^{\cdot}$), hydroxyl ($OH^{\cdot}$), and peroxyl ($ROO^{\cdot}$) radicals and hydrogen peroxide (H_2O_2) , RNS include nitric oxide ($NO^{\cdot}$) and nitrogen dioxide ($NO_2^{\cdot}$). Anti-oxidant constituents from biological sources are of interest for their rules in the maintenance of human health.

Generally it is good to use two different types of assays to investigate the antioxidant activity of plants and their components. The early stage of lipid peroxidation can be monitored by the beta-carotene bleaching method. The DPPH assay is the most frequently used in antioxidant studies of natural products - it is simple and highly sensitive. The ABTS radical assay is widely used to evaluate AOA in food and beverages because it is applicable in aqueous and lipid phases. The Ferric Reducing/Antioxidant Power (FRAP) assay gives fast, reproducible results.

$ABTS^{+}$ Radical Scavenging Assay Procedure

Reagents

1. 50mM Phosphate saline buffer (pH 7.4) - PSB (pH 7.4)
   Dissolve 0.77g monosodium phosphate, 5.18g disodium phosphate and 1.46g sodium chloride adjust volume up to 500 ml using distilled water.
2. 2.5 mM Potassium persulphate solution
   Dissolve 67mg of potassium persulphate in 100ml water
3. Trolox solution (1mg/ml of H_2O or PSB)

Generation of $ABTS^{+}$ Radicals

Dissolve 10 mg of 2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) in 2.5 ml of 2.5 mM pottasium persulphate and incubate at 37°C for 16 h in dark. Mix 1 ml of generated $ABTS^{+}$ radical solution (dark green) with 6 ml of 50 mM PSB (pH 7.4) just before use in the assay.

Sample and Sample Preparation

Water or solvent extracts of the sample can be used in $ABTS^{+}$ assay. All extracts are needed to be filtered and centrifuged before concentrating them to dryness (using freeze dry, vacuum dry or rotary evaporator). Known quantity of the dried sample should be dissolve in water or PSB.

Assay Procedure

1. Add 800 µl PSB (pH 7.4) in to 2 ml vial
2. Add 50 µl of sample and mix well.
3. Add 150 µl of diluted $ABTS^{+}$ solution and mix well
4. Stand for 10 min.
5. Read the absorbance at 734 nm (use PSB as reference)

Experiment Design

Each experiment (for one sample) should include blank, control, treatment, treatment blank and standard (Table 1.). In order to determined IC_{50} (inhibition concentration 50 %) of a sample five different concentration of the sample (within linear region) should be used. Five points standard (Trolox) curve is needed for the calculation of TEAC of the sample. At each concentration at least 3 independent replicates should be used. Generally, one experiment contains 52 vials (Blank 1; standard 5x3; control 6; treatment 5x3; treatment blank 5x3).

Table 1. Experiment Design

	Buffer (µl)	Sample/standard (µl)	ABTS+ (µl)
Blank	1000	0	0
Standard (Trolox)	800	50	150
Control	850	0	150
Treatment	800	50	150
Treatment blank	950	50	0

Calculations

1. **$ABTS^{+}$ radical scavenging % at each concentration.**

   % $ABTS^{+}$ radical scavenging activity = $\frac{C_o - (S_o - Sb_o)}{C_o} \times 100$

   Where;
   C_o is mean absorbance of controls
   S_o is absorbance of sample
   Sb_o is absorbance of respective sample blank

2. **Calculation of IC_{50} [concentration that scavenge 50% of $ABTS^{+}$ radical]**

Plot the % $ABTS^{+}$ radical scavenging activity vs. sample concentration and get the sample concentration at 50 % inhibition.

Note that when the inhibition of highest tested concentration is below 40%, the inhibition activity may not be dose dependent and when the intercept of the curve is >15 % calculation of IC_{50} may lead to a false value.

3. **Trolox equivalent antioxidant capacity (TEAC) of the sample**

Plot the % $ABTS^{+}$ radical scavenging activity vs. Trolox concentrations and interpolate the Trolox equivalent activity of the respective test samples.

Results are expressed as: .....µmole or µg TE / 1g of .....(eg Tea)

Reference:

Prior, R.L.; Wu, X.; Schaich, K. 2005. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food Chem.* 53, 4290-4302

Huang, D.; Ou, B.; Prior, R.L. 2005. The Chemistry Behind Antioxidant Capacity Assays. *J. Agric.Food Chem.*, 53, 1841-1856