

# Aim 37

## To Determine the Total Antioxidant Activity of Plant Extract

### Introduction

Antioxidants are the molecules that have a capacity to reduce oxidative stress by scavenging free radicals and prevent the occurrence of disease caused by free radicals. Free radicals are harmful because they initiate the oxidation of biomolecules such as nucleic acid, proteins, membrane lipids etc which causes cell death and creates oxidative stress. Plants produce number of secondary metabolites that act like antioxidants. These biochemicals are used to control the oxidative stress caused by free radicals and sun radiation.

### Requirements

Beaker, Flask, Shaker, Test tube, Micropipettes, Eppendorf tubes, Water bath, Spectrophotometer, Pipettes etc.

### Reagents

1. 0.6 M Sulfuric acid
2. 28 mM sodium phosphate
3. 4 mM ammonium molybdate

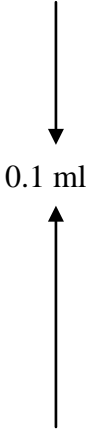

Then mix these solution for preparing solution.

### Procedure

1. Take plant material and dry it under shade.
2. Crush the dried plant material into powder.
3. 30 g of plant powder was soaked in 300 ml of water for 48 hours and regularly shaken at room temperature.

4. Then, it is filtered and the resultant filtrate is evaporated to dryness.
5. Different concentration of (100 to 600  $\mu\text{g/ml}$ ) plant extracts is prepared by dissolving in water.
6. 0.1 ml of each concentration of extracts is combined with 1 ml of reagent solution in an eppendorf tube.
7. Then, tubes containing the reaction solution are incubated at  $95^{\circ}\text{C}$  for 90 min.
8. Finally, tubes are cooled to room temperature and absorbance is recorded at 695 nm of the solution of each extract against a blank (Water, 0.1 ml).
9. Ascorbic acid is used as the standard.

### Observation Table

Sr. No.	Concentration of plant extract ( $\mu\text{g/ml}$ )	Plant Extract	Reagent	Absorbance 695 nm
1.	-	 0.1 ml	 1 ml	
2.	100			
3.	200			
4.	300			
5.	400			
6.	500			
7.	600			

### Formula Used

The percent of total antioxidant activity is calculated using the formula.

$$\% \text{ Total antioxidant activity} = [(Ac-At)/ac] \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in the presence of samples with the extracts.

### **Precautions**

1. Different concentration of plant extract should be prepared with accuracy.
2. Reagents should be mixed carefully.
3. Eppendorf tube should be tightly closed while putting on water bath in a beaker.
4. A solution of eppendorf tube should be cooled before measuring the absorbance.
5. Mark the different concentration of test tube and Eppendorf tubes.