

## EXPERIMENT IV - DETERMINATION OF ANTIOXIDANT ACTIVITIES OF VARIOUS PLANTS

### Chemicals:

- Ethanol
- In order to dissolve DPPH free radical ethanol is used in the experiment. Because DPPH can only be dissolved in an organic medium (especially alcohols),
- DPPH
- As an indication of antioxidant activity, the decentralized electron has the potential to increase violet color. In order to minimize violet color DPPH is used.
- Herbals  
Rosemary  
Thyme

**Principle:** In this experiment radical version of DPPH is being cleaned by redox reaction and antioxidants. DPPH radical turns into a colorless ethanol solution in the presence of antioxidant. DPPH changes colour from purple to yellow.

The principle is based on the cleaning of the radical by redox reactions and the use of antioxidants.

### Procedure:

- 0.1 mM DPPH solution: 0.0038 g DPPH is prepared by dissolving in 100 mL ethanol. Solution should be stored in the refrigerator and in the dark when not in use and should be used in 7-8 hours. The purpose of this experiment is the determination of antioxidative power of ethanolic extracts obtained from various plant sources. For this purpose, each experimental group will bring a different herbal sample. This herbal sample may be a vegetable, fruit or herb, spice, flower, leaf or root. It is preferred to be an edible sample. 1 g of the sample will be weighed. Therefore, it should be avoided to bring large amount of samples.
- Each experimental group takes 1 g of the sample that they brought and then cut it into small pieces. If grinding is required after placing the sample in a mortar or beaker, 5 ml of ethanol is added, and the tissue is grounded for at least 2 minutes with a glass baguette (or pestle). Using a pasteur pipette, the liquid portion is taken into a graduated tube as cleanly as possible and its volume is measured approximately. If there are particles in the solution, centrifuge it 5 minutes at 4000g.
- In this experiment rosemary and thyme are used as herbals.
- 0.3 ml of the sample is taken, and 0.5 ml of ethanol and 1 ml of DPPH solution are added and vortexed thoroughly. Wait for 5 minutes read the absorbance against ethanol at a wavelength of 517 nm.
- Then the sample is diluted with ethanol in various proportions (1/10, 1/50, 1/100 etc.). The absorbance is measured against ethanol at 517 nm wavelength after 10 minutes. These values are  $A_1$  values.

- 1 ml DPPH is added to 0.8 mL ethanol and 517 nm against ethanol 10 minutes later. measured at wavelength. This value is called the  $A_0$  value.
- **%DPPH Values:**  $[A_0 - A_1]/[A_0] \times 100$
- $A_0$  absorbance value is 0.60

Thyme	Absorbance	Rosemary	Absorbance
1/10	0.040	1/10	0.025
1/50	0.010	1/50	0.001
1/100	0.50	1/100	0.2

## **Interpret the results. Which spice and dilution has better antioxidant activity?**

### ***Questions:***

#### **1) What are the beneficial and harmful effects of antioxidant consumption?**

There are several antioxidant systems within the body that help cope with the oxidative stress that results from regular metabolic processes. Antioxidants in diet can also cancel out the cell-damaging effects of free radicals. These antioxidant supplements act in addition to the endogenous systems and their lack can cause several ill-consequences of oxidative stress. There is evidence that some types of vegetables and fruits protect against a number of cancers and other diseases. Large studies have shown that people who took regular antioxidants in fruits and vegetables seemed to have lesser incidence of these diseases. In addition, those who took fewer amounts of antioxidants, or had excessive exposure to pro-oxidants like cigarette smoking etc., had a higher risk of these disorders. For example, oxidation of low density lipoprotein (LDL) in the blood contributes to heart disease. Those taking Vitamin E supplements had a lower risk of developing heart disease.

Some of the antioxidants when taken in excess in diet may cause more harm than good. For example, when a person takes in excessive amounts of strong reducing agents as antioxidants, he or she may develop deficiency of several minerals like iron and zinc. The absorption of these minerals is prevented from the gastrointestinal tract.

Notable examples are oxalic acid, tannins and phytic acid, which are high in plant-based diets. In addition, there may be Calcium and iron deficiencies in persons who take too much phytic acid from beans, legumes, maize and unleavened whole grain bread. Similarly oxalic acid is present in cocoa, chocolate, spinach, turnip and rhubarb and tannins are present in cabbage, tea and beans. Excess of these in diet may prevent mineral absorption. Eugenol, an antioxidant present in oil of cloves, also possesses toxic effects in high levels. Toxicity associated with high doses of water-soluble antioxidants such as ascorbic acid are less of a concern since these can be excreted rapidly in urine. Very high doses of some lipid soluble antioxidants may have harmful long-term effects.