

## EXPERIMENT IV – TOTAL PHENOLIC CONTENT

Polyphenols are compounds where multiple phenol groups are present in each molecule. They are commonly found in plants and are responsible for the pigmentation of plants, such as the colors of leaves in autumn. Polyphenols constitute one of the most important groups of natural antioxidants. Due to these properties, they are believed to have potential benefits for human health. There is evidence suggesting that polyphenols, as antioxidants, reduce oxidative stress (stress caused by reactive oxygen) and thus decrease the risk of cardiovascular disease and cancer. It has also been shown that these compounds delay the onset of Alzheimer's disease. The most common plant-derived phenolic antioxidants include flavonoids, phenolic acids, and tannins. Phenolic acids are divided into two classes: hydroxycinnamic acid and hydroxybenzoic acid derivatives.

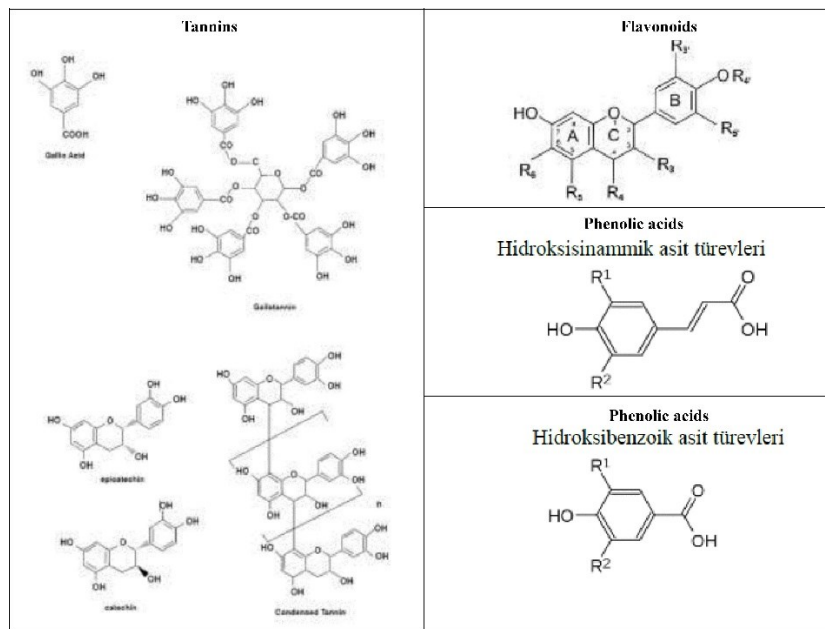


Figure 1: General structures of polyphenols.

### Chemicals:

- Plant extract
- Folin-Ciocalteu reagent
- $\text{Na}_2\text{CO}_3$
- Gallic acid
- Distilled water
- Methanol

## Principles:

Total phenolic substance amounts are determined as equivalent to gallic acid using the Folin-Ciocalteu reagent. The basis of total phenolic substance determination is based on the redox reaction in which phenolic compounds reduce the Folin-Ciocalteu reagent in a basic environment and turn into oxidized form. Folin-Ciocalteu reagent acts as the oxidizing compound here. By measuring the absorbance of the blue color formed by the reduced reagent as a result of the reaction, it is possible to calculate the total amounts of phenolic compounds in the analyzed sample. The color intensity of the resulting complex is directly proportional to the concentration of phenolic substances.

## Solutions:

**Sample: Extraction process:** Weigh 2 grams of plant extract (St. John's wort (*Hypericum perforatum*), lavender (*Lavandula angustifolia*), etc.) and add 20 ml of methanol. The solution is shaken vigorously and filtered. The volume of the filtrate is determined (extract volume). Concentration of plant extract is 0.1 g/mL.

**Folin-Ciocalteu reagent:** Take 2.5 mL of Folin-Ciocalteu reagent from the 2 N stock solution and dilute by adding 7.5 mL of water (1:4). Final concentration of reagent is 0.5 N.

**Gallic acid (To be used as standard):** Prepare 10 mg of gallic acid with methanol in 10 mL. Final concentration of gallic acid 1 mg/mL.

- First standard: 0.4 mL gallic acid stock solution
- Second standard: 0.2 mL gallic acid stock solution + 0.2 mL methanol
- Third standard: 0.1 mL gallic acid stock solution + 0.3 mL methanol

**Na<sub>2</sub>CO<sub>3</sub>:** 17.5 g Na<sub>2</sub>CO<sub>3</sub> is prepared in 250 mL. Final concentration of the solution is %7.

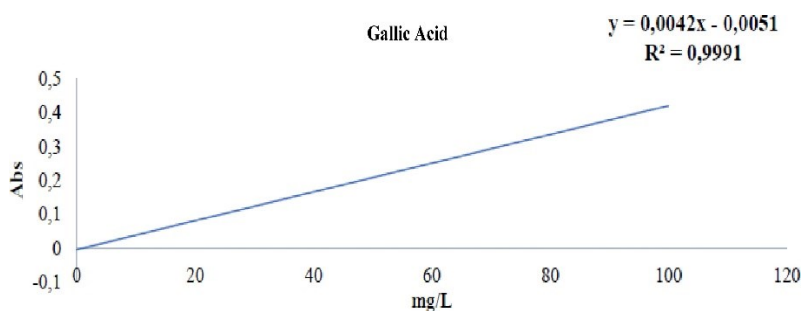
## Procedure:

Gallic acid is prepared with methanol at different concentrations (1:0, 1:1, 1:4), the absorbance is read at 725 nm, and a graph of absorbance versus concentration is drawn. Calculation is made as the amount of phenolic substance in 1 gram of extracts, mg gallic acid equivalent [mg GAE (gallic acid) / g extract].

**Table 1:** Method of total phenolic content

Chemicals	Blank	Standard	Sample
Water	5.6 mL	5.2 mL	5.2 mL
Gallic Acid	-	0.4 mL	-
Sample	-	-	0.4 mL
Folin-Ciocalteu reagent	0.4 mL	0.4 mL	0.4 mL
Na <sub>2</sub> CO <sub>3</sub>		4 mL	
Vortex			
30 min incubation			
Absorbance measurement at 725 nm wavelength			

A report is plotted on millimeter graph paper with absorbance data on the y-axis in absorbance values and concentration data on the x-axis in mg/mL. Subsequently, the concentration is determined by reading the absorbance from the graph. If the graph is to be drawn electronically, the distribution points are plotted, and then a linear trendline is fitted to the points, with the R<sup>2</sup> value and the equation of the line displayed on the graph. Using the equation of the line, the concentration of an unknown sample can be calculated.



**Figure 2:** Example of gallic acid standard calibration graph

The result concentration of plant extract is calculated in “%” using the value read from the graph (Attention: Don’t forget volume of tubes).

### **Interpret the Results:**

**Which plant (or spice) and dilution has better antioxidant activity? Compare these two plants and explain why?**

### **Questions:**

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