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Analysis of bioactive compounds in fresh and dried food products using destructive methods

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Topics

- The analysis of bioactive compounds in fresh and dried Indian gooseberry
    - Vitamin C using HPLC
    - Total phenolic content using Folin-Ciocalteu reagent
    - DPPH radical scavenging

Indian gooseberry / มะขามป้อม (Makham Pom)

- Contain high ascorbic acid (vitamin C)
- Contain inositol, tannin, gallic acid, quercetin and kaempferol

Fresh Indian gooseberry

Dried Indian gooseberry

The analysis of Vitamin C using HPLC

Methodology

Step 1: Reagent preparation

Step 2: Sample preparation

Step 3: HPLC measurement

Equipment and apparatus

HPLC apparatus

Equipment and apparatus

Balance

Sonicator bath

Herb grinder

Equipment and apparatus

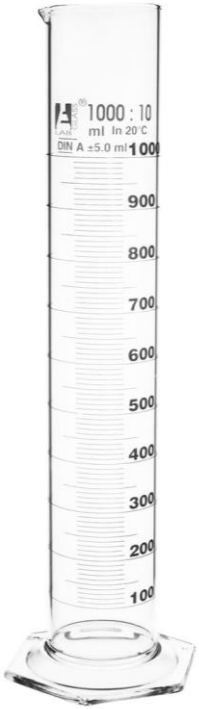
Vortex mixer

Homogenizer

Food blender

Equipment and apparatus

Volumetric flask



Measuring cylinder

Funnel

Erlenmeyer flask

Equipment and apparatus

Vacuum filtration apparatus

Filter cloth

Equipment and apparatus

syringe

nylon syringe filter

Whatman filter paper

Reagents and chemicals

meta phosphoric acid

Distilled water

Step 1: Reagent preparation

4.5% w/v meta-phosphoric acid solution

- Weigh 22.5 g of meta-phosphoric acid
- Dissolve it with distilled water to a volume of 500 mL in a volumetric flask

Step 2: Sample preparation

Fresh Indian gooseberry sample

Step 2: Sample preparation

Fresh Indian gooseberry sample

- Blend the fruit with water at 1: 5 ratios (w/v) using food blender
- Squeeze the sample juice using filter cloth
- Mix the juice 0.5 mL with 9.5 mL of 4.5% meta-phosphoric acid solution
- Filtrate using 0.45 µm-nylon filter

Step 2: Sample preparation (cont.)

Dried Indian gooseberry sample

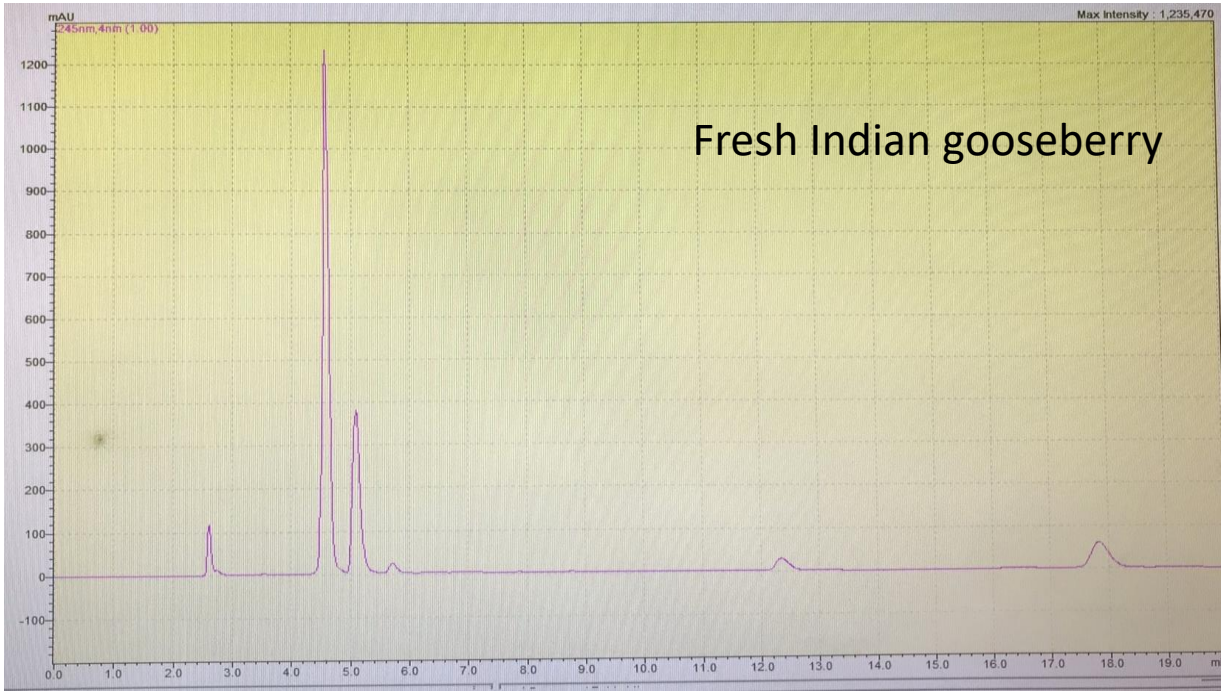
- Grind the fruit into powder using herb grinder
- Mix the powder 1 g with 4.5% meta-phosphoric acid 50 mL
- Sonicate for 20 min
- Filtrate using vacuum filtration
- Filtrate using 0.45 µm-nylon filter

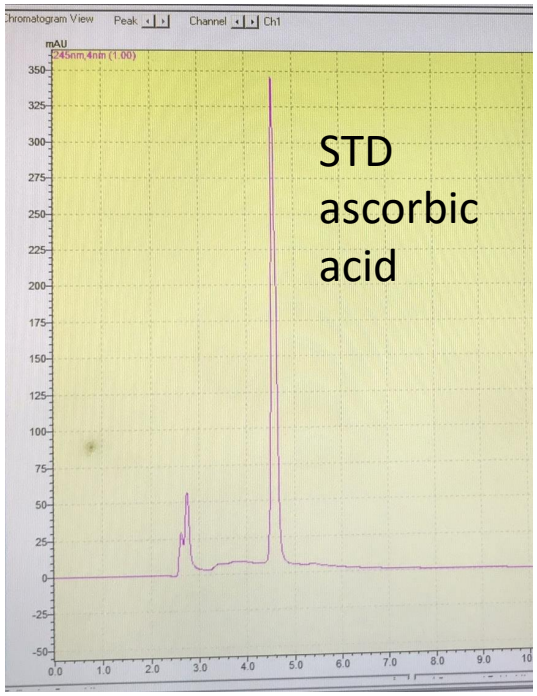
Step 3: HPLC measurement

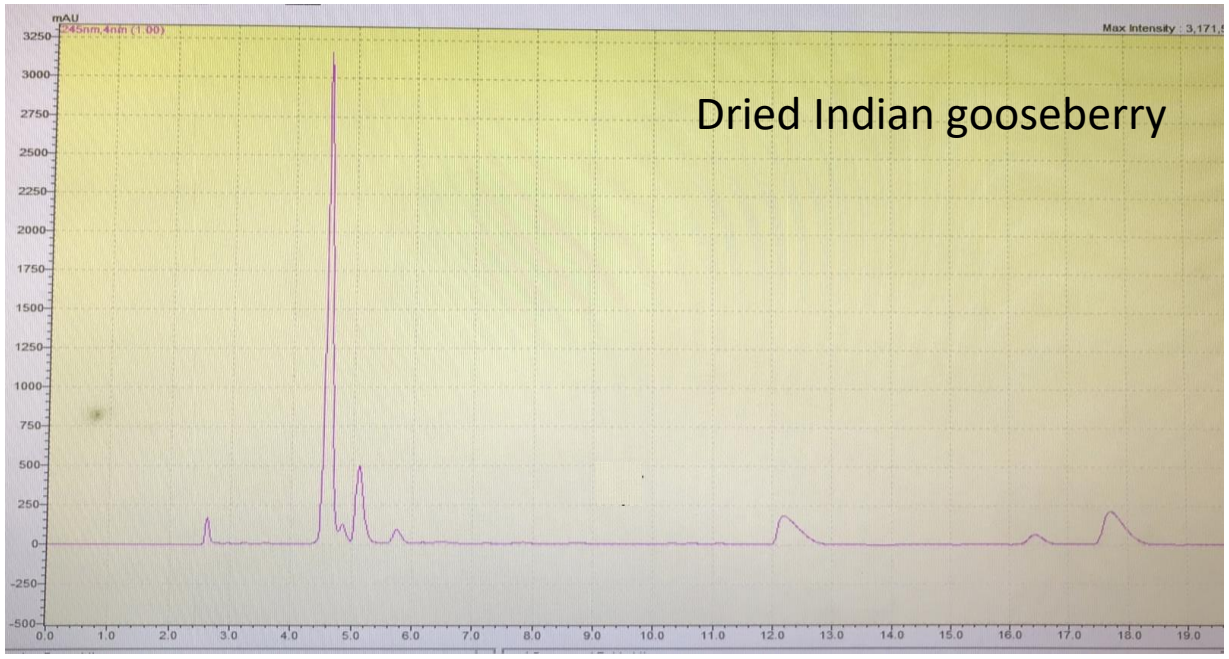
HPLC condition

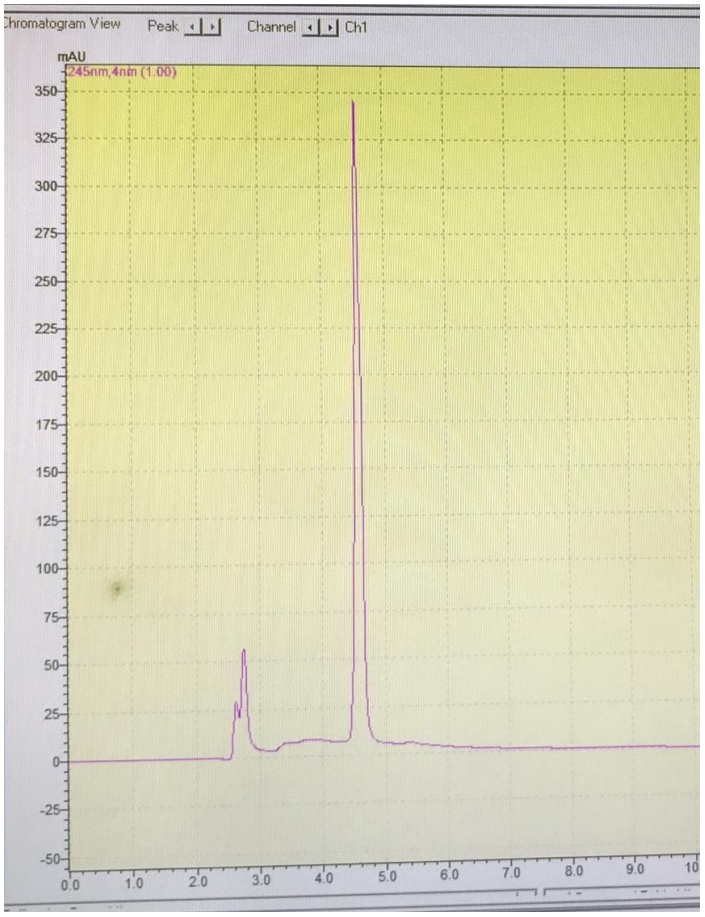
- C18 Column, size 250 mm
- Mobile phase: 0.01% w/v sulfuric acid, adjust pH to 2.6 with 0.1 N NaOH at a flow rate of 1 mL/min.
- Sample Volume: 20 µL
- Detector: UV at 245 nm

HPLC-Chromatogram

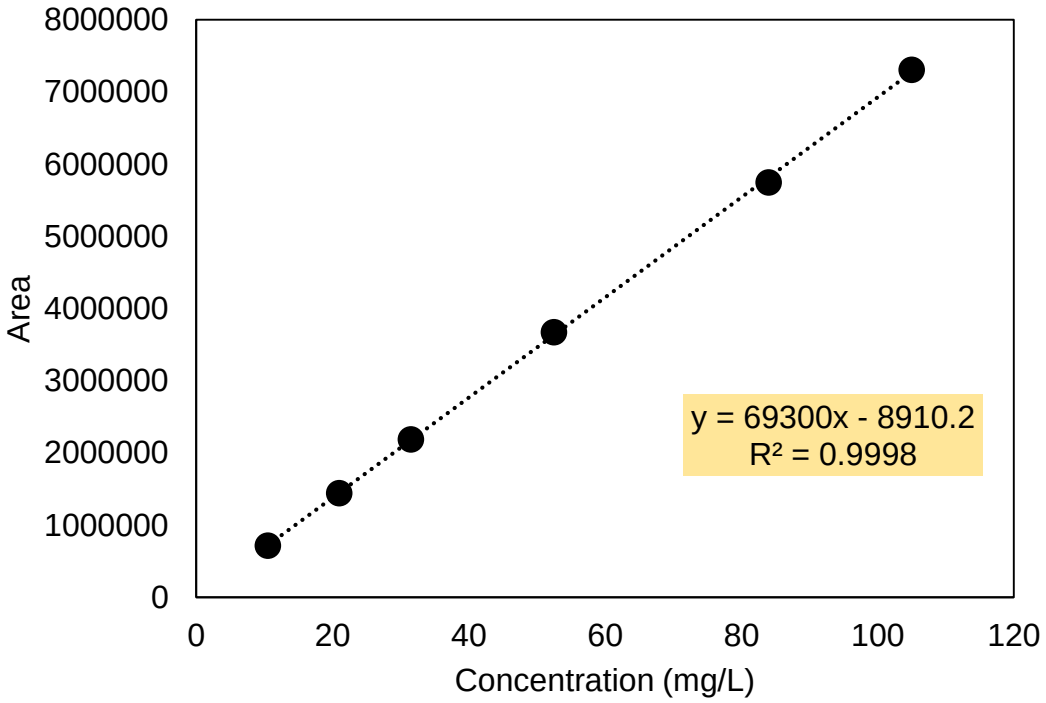








Ascorbic concentration (ppm)	Peak area at 245 nm
100	7302255
80	5743345
50	3669652
30	2182823
20	1438125
10	712176



The analysis of total phenolic content using Folin-Ciocalteu reagent

Methodology

Step 1: Reagent preparation

Step 2: Sample preparation

Step 3: Colorimetric assay

Step 1: Reagent preparation

Folin-Ciocalteu reagent

- dilute the reagent using distilled water at ratio of 1/10 v/v

Sodium carbonate 7.5% (w/v)

Gallic acid 20-100 mg/L

Step 2: Sample preparation

Fresh Indian gooseberry sample

- Blend the fruit with water at 1: 5 ratios (w/v) using food blender
- Squeeze the sample juice using filter cloth
- Mix the juice 0.5 mL with 9.5 mL 80% methanol

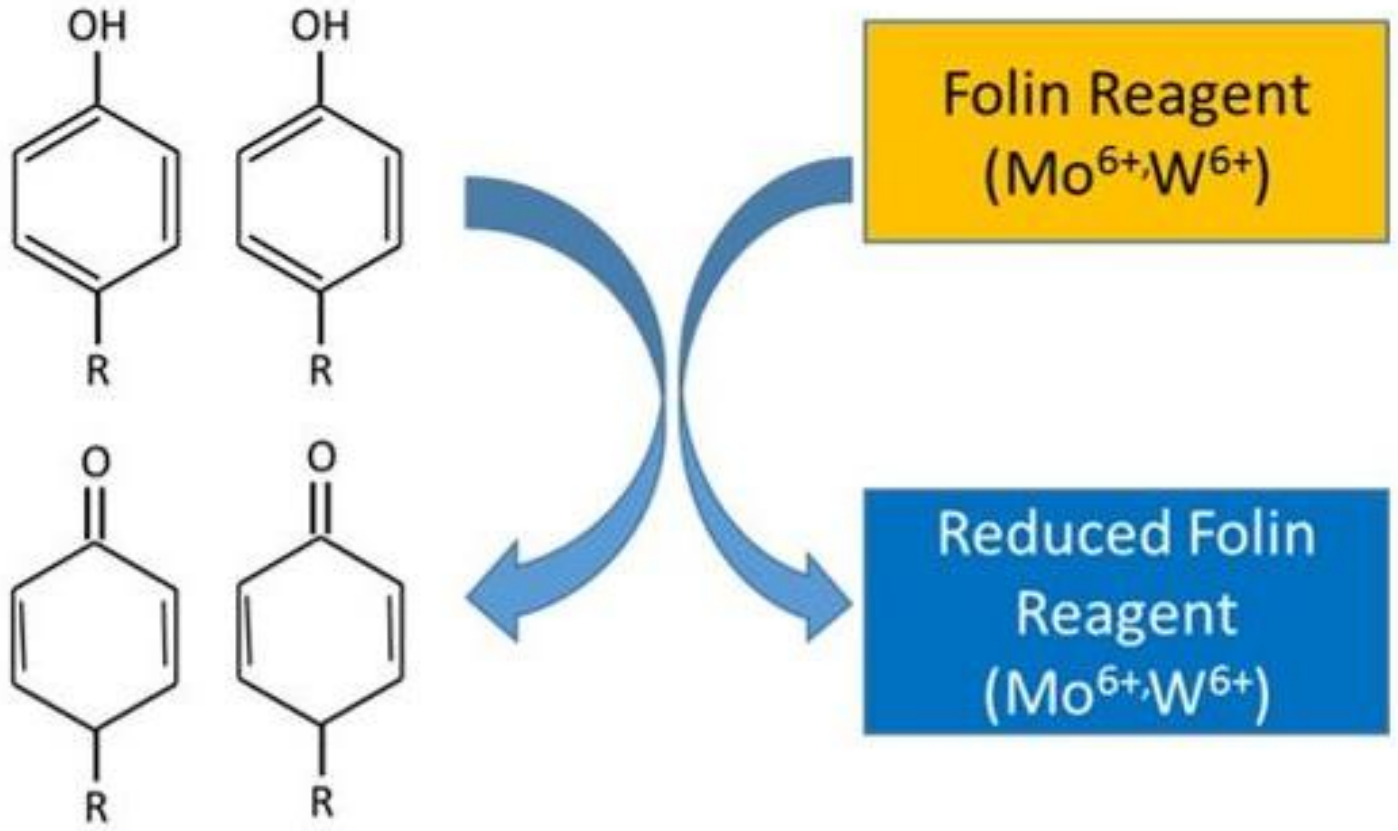
Step 2: Sample preparation (cont.)

Dried Indian gooseberry sample

- Grind the fruit into powder using herb grinder
- Mix the powder 1 g with 80% methanol 50 mL
- Sonicate for 20 min
- Filtrate using vacuum filtration

Step 3: Colorimetric assay

- Pipet 0.3 mL of the sample into the test tube
- Add Folin-Ciocalteu reagent 1.5 mL, mix well
- Set aside for 6 minutes.
- Add 1.3 mL of 7.5% sodium carbonate solution and mix well
- Put it in the dark for 30 minutes


OH
OH
R
R
O
O
R
R
Folin Reagent
(Mo6+,W6+)
Reduced Folin
Reagent
(Mo6+,W6+)

Step 3: Colorimetric assay (cont.)

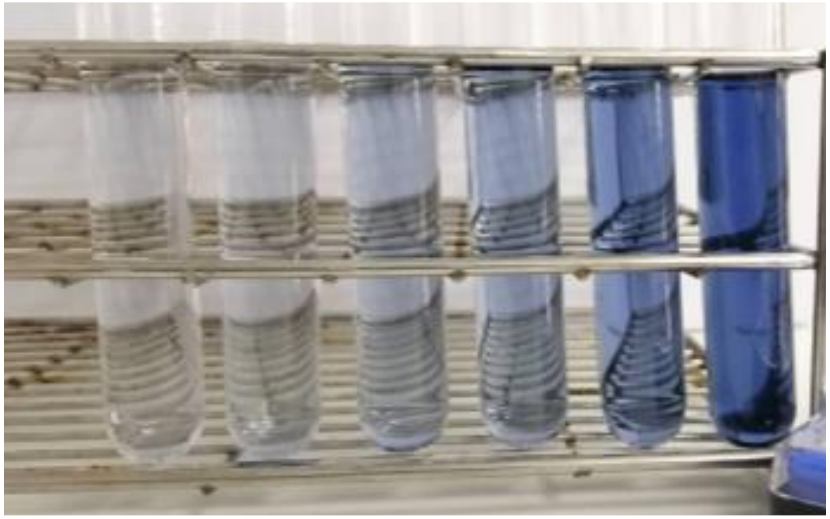
- Measure the absorbance at a wavelength of 760 nm

Spectrophotometer

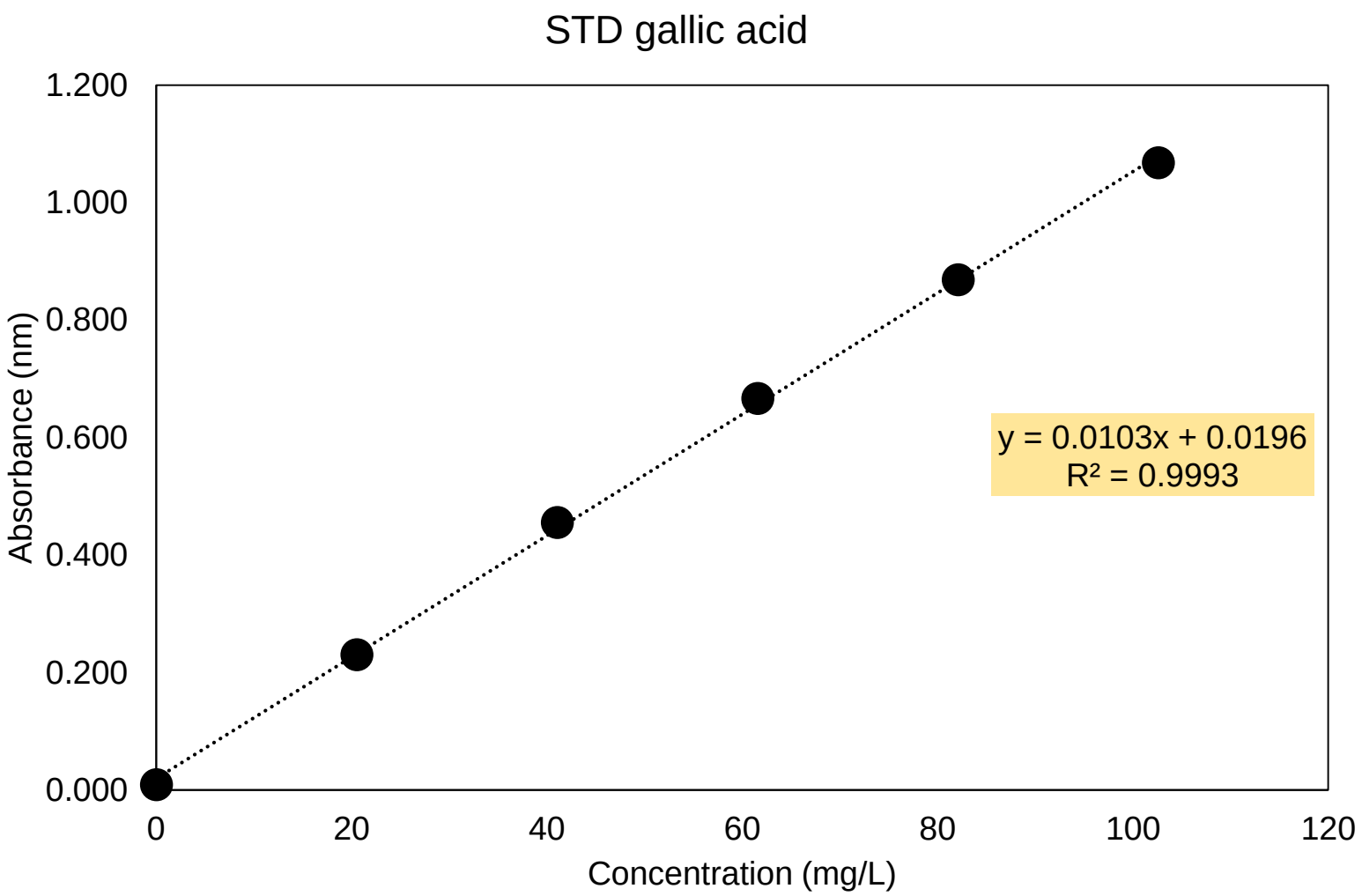
Step 3: Colorimetric assay (cont.)

- Calculate the total amount of phenolic compounds compared with Gallic acid standard graph

Gallic concentration (mg/L)	Absorbance value
0	0.009
20	0.230
40	0.455
40	0.667
60	0.869
100	1.068



Color shading of the gallic acid solution (the referent phenolic compound) after reaction with Folin-Ciocalteu reagent


STD gallic acid
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Absorbance (nm)
0
20
40
60
80
100
120
Concentration (mg/L)
y = 0.0103x + 0.0196
R² = 0.9993

The analysis of DPPH radical scavenging

Methodology

Step 1: Reagent preparation

(DPPH 60 μM in methanol)

Step 2: Sample preparation

(as same as TPC)

Step 3: Colorimetric assay

Step 3: Colorimetric assay

- Mix 0.1 mL of the sample with 3.9 mL DPPH solution in the test tube, mix well
- Put it in the dark for 30 min
- Measure the absorbance at a wavelength of 517 nm
- Calculate the DPPH inhibition

DPPH solution

DPPH solution + Sample

$$\text{DPPH inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the DPPH solution and A_1 the absorbance of the DPPH solution with the sample.