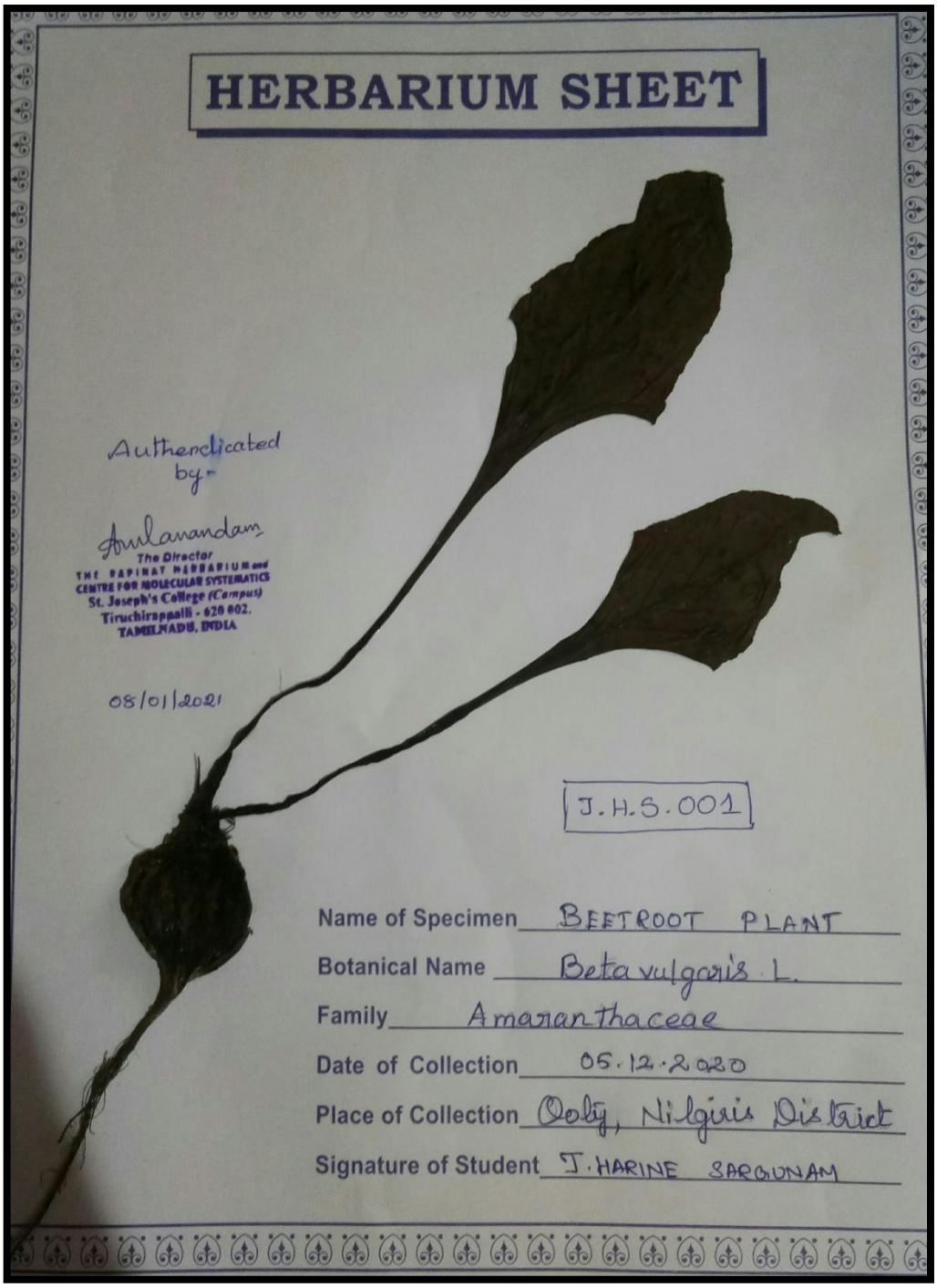
## APPENDIX I

## *BETA VULGARIS* L. PLANT TAXONOMIC AUTHENTICATION



## APPENDIX II

## DPPH ASSAY

**PRINCIPLE:**

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was the oxidizing radical to be reduced by the antioxidant (AH) present in the given sample. The whole reaction was indicates as,

$$DPPH^{+} + AH \longrightarrow DPPH\text{-}H + A^{+}$$

The disappearance of the DPPH radical absorption at 517nm by the action of antioxidants is measured spectrophotometrically in the methanolic solution until the absorbance remains constant.

**REAGENTS REQURIED:**

1. 80 % methanol
2. DPPH (Diphenyl -2-Picryl Hydrozyl was added with 250 ml of methanol (0.1mM).
3. Standard:

   Stock solution: 100mg of Butylated Hydroxy Toluene was dissolved in 100ml of methanol. 100ml working standard was prepared by adding 10ml of stock solution to 90ml methanol. Different concentration of 0.1, 0.2, 0.3, 0.4, 0.5ml of the standard
   were obtained by appropriate dilution with DPPH.

**PROCEDURE:**

1. 0.1ml of the freshly prepared sample was taken in test tube.
2. 6ml of DPPH solution was added.
3. The test tubes were kept in BOD for one hour at 35°C.
4. The O.D of the solution was read spectrophotometrically at 517n.m.
5. The O.D of DPPH solution without sample addition was also read.
6. The difference in the O.D of DPPH solution and DPPH solution + sample was calculated.
7. The decrease in OD with sample addition is used for calculation of the antioxidant activity.

**STANDARD CURVE**

Butylated Hydroxy Toluene was taken as standard. Various concentrations were prepared and added to DPPH solution. The decrease in O.D was plotted against concentration of standard. The concentration of samples was calculated using the standard curve.

## APPENDIX III

## ESTIMATION OF TOTAL PHENOLS

### PRINCIPLE:

Phenols react with and oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent under alkaline conditions and result in the formation of a blue colored complex, the molybdenum blue which is measured at 660 n.m.

### REAGENT REQUIRED:

1. 80% Ethanol ($C_2H_5OH$)
2. Folin and Ciocalteau's Phenol reagent (1N)
3. 20% Sodium Carbonate ($Na_2CO_3$)
4. Standard Phenol Solution:

100 mg of gallic acid was dissolved in 100 ml of distilled water in volumetric flask to prepare a stock standard. Working standard was prepared by dissolving 10ml of stock standard in 90ml distilled water. Test tubes of 0.4 , 0.8, 1.2, 1.6, 2 ml concentration of standard were obtained by appropriate dilution with Folin-Ciocalteau reagent and sodium nitrate.

### PROCEDURE:

1. 0.5ml of freshly prepared sample was taken in the test tubes.
2. 8ml of distilled water was added to all the tubes.
3. 0.5ml of Folin's Ciocalteau Reagent added to all the tubes.
4. All the tubes were kept in B.O.D for incubation at 40°C for 10 minutes.
5. Then, 1ml of Sodium Carbonate solution was added to all the test tubes.
6. Then, the tubes were kept in the dark for incubation for one hour.
7. The color so developed was read spectrophotometrically at 660 n.m.

### STANDARD CURVE

Standard curve was drawn using Gallic acid as standard. Different concentrations of Gallic acid were prepared and O.D was read at 660 n.m. in a Shimadz spectrophotometer. The concentrations of samples were calculated based on the standard curve.

## APPENDIX IV

## ESTIMATION OF TOTAL FLAVONOIDS

**Principle:**

Total flavonoids present in the given sample were measured by spectrophotometrically. The yellow color complex formed by the addition of Aluminium Chloride will form an Aluminium complex which is read at 510 n.m.

**Reagents:**

1. Sodium Nitrate ($NaNO_2$) Solution (5%)
2. 10% Aluminum Chloride solution ($AlCl_3$)
3. Sodium Hydroxide (NaOH) – 1M
4. Standard Solution:
   100 mg of quercetin is dissolved in little amount of distilled water and the volume is made up to 100ml with distilled water. Different concentrations of 0.5, 1, 1.5, 2, 2.5 ml of the standard were obtained by appropriate dilution with distilled water. The concentration of the solution will be 100µg/mL.

**Procedure:**

1. 1.5ml of aliquot of approximately diluted sample was added to 3.5mL of distilled water at zero time.
2. 0.3mL of 5% Sodium Nitrate was added to the tubes.
3. After 5 minutes, 0.3mL of 10% Aluminum Chloride was added to all the tubes.
4. At $6^{th}$ minute, 2mL of 1M Sodium Hydroxide was added to mixture.
5. Immediately, the contents of the reaction mixture were diluted with 2.4mL of distilled water and mixed thoroughly.
6. Absorbance of the mixture was determined at 510n.m versus a prepared blank immediately.
7. Quercetin was used as the standard compound for quantification of total flavonoids as mg per 100g of edible portion.

## APPENDIX V

## PROCEDURE FOR PESTICIDE ANALYSIS USING GCMS

### Sample preparation

The methods for extraction of pesticides and clean up of environmental samples are extremely important for their quantitative determination in the matrices of interest. The proper techniques for powerful chopping devices help in achieving good sample homogeneity and to ensure that a 10g sub-sample is representative for the analysis.

### Extraction/partitioning

Homogenized sample (10 g) is taken into 40 ml Teffon centrifuge tube. To it 20ml acetonitrile (MeCN) is added with the dispenser and tightened with screw cap. It is shaken vigorously for 1 min by using vortex mixer at maximum speed. 4g anhydrous $MgSO_4$ and 1g NaCl are added and mixed on a vortex mixer immediately for 1 min. The action is performed immediately to prevent formation of $MgSO_4$ conglomerates. To it 50 µl standard solution is added, mixed on a vortex mixer for another 30 sec and extract is centrifuged (or batch of extracts) for 10 min at 6000 rpm.

### Dispersive SPE clean up

Transfer 4ml aliqot of upper MeCN layer into 15ml micro centrifuge vial containing 100 mg PSA sorbent and 600 mg anhydrous $MgSO_4$, cap tightly. Shake by hand or with Vortex mixer for 30 sec. Centrifuge extracts (or batch of extracts) for 5 min at 3000 rpm to separate solids from solution and transfer the resulting cleaned extract of 2.0ml to a graduated 15ml borosilicate glass turbovap tube. The extract is evaporated to dryness under a gentle stream of nitrogen in a low volume concentrator by using the Turbovap LV set at $40^{I}$ C. The residues are then dissolved in 1ml of hexane and the extract is transferred to an auto sampler vial and an aliquot is analyzed using GC and GC-MS analysis.

## APPENDIX VI

## PROCEDURE FOR PHYTOCHEMICAL SCREENING

**1. Test for Alkaloids (Wagner's reagent)**

A fraction of extract was treated with 3-5 drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

**2. Test for Flavonoids (Alkaline reagent test)**

2 ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless or addition of dilute hydrochloric acid, indicates the presence of flavonoids

**3. Test for Phenols (Ferric chloride test)**

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

**4. Test for Phlobatannins (Precipitate test)**

Deposition of a red precipitate when 2mls of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

**5. Test for Amino acids and Proteins (1% ninhydrin solutionin acetone)**

2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

**6. Test for Sterols (Liebermann-Burchard test)**

1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H2SO4 and observed for the test

**7. Test for Saponins (Foam test)**

To 2mls of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

**8. Test of Tannins (Braymer's test)**

2 mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish color solution.

**9. Test for Terpenoids (Salkowki's test)**

1 ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

**10. Test for Quinones**

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration)

**11. Test for Oxalate**

To 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black coloration indicates presence of oxalates

## APPENDIX VII

## ESTIMATION OF HYDROGEN PEROXIDE SCAVENGING CAPACITY

**Procedure:**

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined the phosphate buffer without hydrogen peroxide

**Calculation:**

The percentage of hydrogen peroxide scavenging= % Scavenged $[H_2O_2]=[(A_c - A_s) / A_c] \times 100$.

## APPENDIX VIII

## ESTIMATION OF REDUCING POWER

Substances which have reduction potential react with potassium ferricynaide to form potassium ferrocynaide, which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm. An increase in the reduction of ferric to ferrous ion increases the absorbance indicating the reducing ability of ethanolic leaf extract.

***Reagents***

1. Phosphate buffer - 0.2 M, pH 6.6
2. Potassium ferricyanide - 1% in water
3. Trichloroacetic acid (TCA) - 10% in water
4. Ferric chloride - 0.1 w/v in water

***Procedure***

Varying concentrations of ethanolic leaf extract of *P. guajava* (250, 500, 750, 1000 and 1500 g) in double distilled water was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, after which, 1.5 mL of TCA was added and centrifuged at 3000xg for 10 min. From all the tubes, 0.5 mL of supernatant was mixed with 1 mL of distilled water and 0.5 mL of ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer. The increased absorbance of the reaction mixture indicated increasing reducing power. Incubation with water in place of additives was used as the blank.

## APPENDIX IX

## PROCEDURE FOR NUTRIENTS AND MINERAL ESTIMATION

### A. Determination of Moisture Content (AOAC 200)

The total moisture content of the sample was determined as described by AOAC (2000)

About 10g sample was taken in petriplate. The sample was dried in hot air over at 130°C for two hours. The petriplate along with the sample was taken out and allowed to cool to room temperature in a dessicator. The weight of the sample after was measured and moisture content was calculated according to the following formula

$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

### B. Determination of Ash Content (AQAC 2000)

The total ash content of the sample was determined as described by AOAC (2000). About 5g sample was taken in a crucible. The sample was kept in a muffle furnace at 555°C for 5hrs. The crucible was allowed to cool to room temperature in a dessicator. The final weight was taken and the percentage weight of the ash content was calculated using the formula

$$\text{Ash content (\%)} = \frac{\text{Ash Weight}}{\text{S. wt}} \times 100$$

$W_1$ – Initial weight of the sample $W_2$ – Final weight of the sample S.wt– weight of the sample

### C. Estimation of Fibre (Maynard 1970)

Estimation of crude fibre: For estimation of crude fibre, 2g of dried tissue was boiled in 200 ml of sulphuric acid (1.25% w/v) for 30 min. Then it was filtered through muslin and washed with boiling water until the filtrate was no longer acidic, further boiled with 200 ml of sodium hydroxide (1.25% w/v) solution for 30 min, filtered through muslin, washed with 25 ml absolute alcohol. Remove the residue and transfer to ashing dish (Pre weighed dish $W_1$) and dried for 2 hours at 130±2°C. Cool in a desiccators and weigh ($W_2$). Ignite for 30 minutes at 600°C± 15°C. Cool in a desiccators and reweight ($W_3$).

Calculation (g) =

$$\frac{\text{Loss in weight on ignition (W2-W1) – (W3-W1)}}{\text{Weight of the sample}} \times 100$$

**D. Estimation of Protein by Kjeldahl Method (AOAC 2000)**

The total protein in the sample is determined according to the Kjeldhal Method as describes in AOAC (2000). The method consists of three steps : 1) DIGESTION

0.5g of the sample was weighed and put in the digestion tube without touching the side of the tube. Then add 4g of the catalyst mixture (potassium sulphate) and copper sulphate in the ratio aspirator on and set the recommended temperature (4200C). After required temperature is reached digest the sample for 40 min. Switch the digestion unit off and let the digestion tube in the stand cool down in a separate place.

**Distillation**

Dilute the cool by adding 50ml of distilled water and place the tube into the distillation unit. Place a titration flask containing 4% boric acid and two drops of methyl red dye indicator into the unit. It takes for 9minutes.After the digestion is connected to a received flask by a time. The solution in the digestion flask is then made alkaline by the addition of sodium hydroxide which converts the ammonium sulphate into ammonium gas. The ammonium gas that is formed is liberated from the solution and makes out of the digestion flask and flows into the receiving flask which contains an excess of baric acid. The low pH of the solution in the ammonium ion and simultaneous converts the boric acid to the borate ion.

**Filteration:**

The nitrogen consent is then estimated by titration of the ammonium borate formed with standard HCL of 0.1N using a suitable indicator to determine the end point of the reaction. The concentration of hydrogen ions required reaching the end point is equivalent to the concentration of nitrogen that was in the original food sample. The following equation can be used to determine the nitrogen concentration of the sample.

$$\%N - \frac{14.01 \times 0.1 \times (T.V - B.V)}{W \times 100} \%Protein = \%N \times \text{conversion factor} \times 100$$

T. V = titrate value

B.V = blank value

W = weight of the sample

Conversion factor for protein powder is 6.25

Atomic weight of Nitrogen – 14

## E. Formula for Estimation of Fat (Soxhlet Method) AOAC (2000)

**Principle**

Weight the sample in a filter paper and fold it. Keep it in the thimble and then keep it in the oil flask. Fill the oil flask with 60ml hexane. Keep oil flask in the soxhlet apparatus for 75°C for 35 min or till the beep sound. (For soaking the solvent). Later allow it for 30 minutes at 190°C for fat to get separated. Later remove the thimble and keep the oil flask in the hot air oven. (in oil flask fat gets separated).

$$\text{Fat\%} = \frac{\text{final weight of oil flask} - \text{initial weight of oil flask}}{\text{Sample weight}} \times 100$$

**Determination of Calcium**

**Principle**

This standard prescribes the methods for the volumetric determination of calcium and magnesium using ethylenediamine tetraacetic acid (EDTA).

**Procedure**

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the @H to 12’5 to 13 with 20 percent sodium hydroxide solution (4’6) using a pH indicator paper or preferably at pH meter. Add approximately 0’1 g of Patton and Reeder’s indicator mixture and stir to dissolve. Titrate with 0’01 M EDTA solution from a burette, till the color changes from red to pure blue free from any violet tinge. Towards the end point, the color turns a violet which on further dropwise addition gives the pure blue end point. It is easier to note the end point by comparing the color with that of a previously titrated solution having a pure blue end point, where a slight excess of EDTA has been added.

**Calculation**

$$\text{Calcium (as Ca) percent by mass} = \frac{V_1 \times 0.040\,08}{M}$$

$V_1$ = volume in ml of EDTX solution consumed in titration; and M = mass in g of the sample in the solution taken for the test.

## F. Analysis of Iron in Foods

**Principle**

The deep red color of the iron (III) thiocyanate ion is directly related to the concentration of iron (III) originally present in the solution.

**Materials/Equipment:**

0.001 M $Fe(NO_3)_3$ solution in 0.1 M HCL, 0.1 M KSCN, 0.1 M HCl, 2.0 M HCl, Distilled water food sample, crucible, ring stand, ring, Bunsen burner, funnel, filter paper, beakers, 20x150 mm test tubes/test rack, Spectrophotometer or UV-VIS, cuvettes

**Procedure:**

1. Preparing the Standards:
2. Standard solutions were prepared in five test tubes with **0.0101 M $Fe(NO_3)_3$ (mL) and water mixture at 0.00, 0.25, 0.05, 0.75, 1mM/L concentration.**

Add 2.5mL of 0.1 M KSCN to each test tube. Mix well. A red color should result from the formation of the $FeSCN^{2+}$ ion.

**Preparing the Food samples**

1. Weigh out about 2.5 g of the solid food and place in a crucible.
2. Heat the crucible with a hot burner flame until the food sample has turned to ash. This should take approximately 5-20 minutes depending on the food sample used.
3. Remove the burner and allow the ash to cool. When cool, transfer to a small beaker.
4. Add 10mL of 2.0 M HCl and carefully stir for one minute. Add 10 mL of distilled water. Stir
5. Filter the mixture, collect the filtrate. Add 2.5mL of 0.1 M KSCN. Mix well.

**Finding the Absorbance**

6. Use a spectrophotometer at a wavelength of 458 nm or use the fixed wavelength (458
   nm) on the UV-VIS
7. Place each standard solution and food solution into a separate cuvette.
8. Measure and record the absorbance of each solution.

**Calculation**

1. Prepare a standard curve (Beer's Law) of the standard concentrations vs. absorbance.
2. Use the standard curve to determine the iron (III) concentration of the food samples you tested.
3. Convert the concentration from mM/L to mg/L of $Fe^{3+}$.

## G. Estimation of Vitamin C – Titration Method

### Principle

The method devised to determine vitamin C is the redox titration with 2,6-dichlorophenol indophenols (DCIP). The oxidation of ascorbic acid yields dehydroascorbic acid. This reaction is a redox reaction in which vitamin C (ascorbic acid) is oxidized to dehydroascorbic acid and DCIP is reduced to the colorless compound DCIPH2. DCIP is blue in neutral solution and pink in acidic solution. The reduced form is colourless, so the endpoint of the titration is the appearance of a faint pink colour. All reagents used in the analysis were of analytical grade and all titrations were done in triplicates.

### Procedure

The vitamin C present in the samples was extracted with 10% oxalic acid and thereafter determined by titrimetric method using 2.6-dichlorophenol indophenols acid. The method involved preparing a stock standard solution and a working standard solution. The stock standard solution was prepared by dissolving 100mg ascorbic acid and 100ml of 4% oxalic acid solution. The working standard solution was prepared by diluting 10ml of the stock solution to 100ml with 4% oxalic acid (100µg/ml). From this solution 5ml was pipette into a 100ml conical flask and 10ml of 4% oxalic acid was added. This was titrated against the dye and the end point was indicated by the appearance of a pink coloration that persisted for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. Then 5g of the sample was extracted and made up to 100ml volume using 4% oxalic acid and centrifuged. 5ml of the supernatant was pipette and 10ml of 4% oxalic acid was added and titrated against the dye and the volume was recorded. Ascorbic acid content was expressed in mg/100g of the sample.

$$\text{Ascorbic acid content (mg/100ml of sample)} = \frac{0.5\text{ml}}{V_1\text{ ml}} \times \frac{V_2\text{ml}}{5\text{ml}} \times \frac{100\text{ml}}{\text{Sample wt}} \times 100$$

## APPENDIX X

## ESTIMATION OF FLAVONOIDS USING HPLC

### Standard preparation

Standard stock solutions of five phenolic compounds were prepared in methanol, at concentrations of 0.0420, 0.434, 0.400, 0.402 and 0.402 mg.mL-1 for gallic acid (GA), Catechin (CA), rutin (RU), ellagic acid (EA), quercetin (U). All standard solutions were filtered through 0.45 mm membrane filter (Millipore), and injected by autosampler.

### Sample preparation

The extraction was carried out using 2g of leaf powder with 50mL of 95% ethanol under 80KHz, 45° C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 mL mobile phase. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the sample was injected into HPLC by autosampler. Detection wavelength was 280 nm. High Performance Liquid chromatography was performed with a Shimadzy (Kyoto, Japan) system consisting of a column oven (model CTO-10ASVP), a UV-visible diode-array detector (model SPD-M10 Avp), a degasser (model DGU14 A), and a liquid chromatography pump (model LC-10AT-VP); Shimadzu software was used to calculate peak areas. The sample (10 µL) was injected into the HPLC with a syringe (Hamilton, Reno, NV, USA). The HPLC column used was a reversed-phase C18 (150 mm x 4.6mm, 5 µm; #504955) from Supelco (Bellenfonte, PA, USA). T.flow; 1.000 mL/min B.Conc; 0.0, B Curve: 0.0, P.Max: 400.0 kgf/cm2, P.Min: 0.0 kgf/cm2, CTO-10ASvp, Temperature: 40°C, SPD-10Avp(DetA), Lamp: D2, Polarity: +. The name of the compounds were ascertained and the compounds were quantified using peak area normalization.

## APPENDIX XI

## SCREENING FOR SECONDARY METABOLITIES GCMS INTERPRETATION

### GCMS Instrumentation

The chemical compositions of 1 ml were investigated through Gas Chromatography Mass Spectrometry/Electron Ionization (GC-MS/EI) mode. The GC-MS is an Perkin Elmer GC Claurus 500 system which is interfaced to a Mass Spectrometer equipped with a Elite-5 MS fused silica capillary column (30m x 0.25mm x 0.25µm df) composed of 5% Diphenyl and 95% Dimethyl poly siloxane. In Mass Spectrometry, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 2µl was employed (split ratio of 10: 1). Injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/ min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV.A scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms were Turbo Mass Version 5.2.0.

### Interpretation of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 1,62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components store in the NIST library. The main criteria for selection of suitable ions for an identification of compound should have a high peak area (> 0.05%) and should be unique and be well resolved from other ions with the same mass to charge ratio (m/z) in the defined time window. Identification of the compounds indicated by the library search program as being more than 80% and viewed as being likely hits. The name, molecular weight and structure of the components of the test materials were ascertained. The compounds were quantified using peak area normalization.

## APPENDIX XII

## ESTIMATION OF VITAMIN A USING HPLC

### Standard Preparation:

Standard of beta carotene (1g enclosed in vial) was obtained from Merck. Stock solution of beta carotene was prepared by taking 10mg in 100 ml n-hexane. The concentration of stock solution was equal to 100 ppm.

### Sample Preparation:

10 g of sample was homogenized in 30 ml of acetone and then 0.1% (BHT) solution in acetone was added as an antioxidant. The resulting extract was filtered through Buchnar's funnel. The residue was washed twice with acetone till it become colorless. The residue was discarded and filtrate was combined with 20gm of anhydrous sodium sulphate. The anhydrous sodium sulphate was removed through filtration and the volume of extract was reduced by rotator evaporator. The extract was transferred quantitatively to 100 ml volumetric flask and the volume was made up to the mark with acetone and water, so that the final extract contain 80% of acetone.

### Instrumentation

HPLC was calibrated by running mobile phase (Acetonitile, dicholormethane and methanol by the ratio of 70:20:10 respectively) at the rate of 2ml per minute. Wave length was fixed at 452 nm. The pressure of the column was kept 1800-2000 PSI. Each sample extract in 80% acetone was used for HPLC assay like standard. The 20µl of leaf sample was taken by micro liter syringe. The peak was automatically identified and quantified by comparing its retention time of the sample with the standard retention time.

## APPENDIX XIII

## ESTIMATION OF VITAMIN E USING HPLC

**Standard Preparation:**

Standard vitamin E (DL-a-tocopherol) were purchased from Sigma (St.Louis , MO, USA), These vitamins were prepared in n-butanol. All other reagents were also from Sigma.

**Sample Preparation:**

One gram of the sample was precisely weighed and transferred to a 10-ml screw-capped extraction. Four ml of n-hexane was added to the tube and the tube was flushed with a steam of Nitrogen to protect vitamins from air exposure before capping. The mixture was shaken on a vortex mixer for 0.5 min, and rested for 5 min, and shaken another half minute. After centrifugation at 4000 rpm for 5 min, 1 ml of that supernatant was transferred to a 1.5ml vial and evaporated under nitrogen to remove the solvent. The residue was re-dissolved in 0.3 ml n-butanol particle size, ratio of sample to reagent, extraction before being injected into the HPLC system.

**Instrumentation:**

High Performance Liquid Chromatography was performed with a Shimadzu (Kyoto, Japan) system consisting of a column oven (model CTO-10ASVP), a UV-visible diode-array detector (model SPD-M10 Avp), a degasser (model DGU14 A), and a liquid chromatography pump (model LC-10AT-VP); Shimadzu software was used to calculate peak areas. The sample (10µL) was injected into the HPLC with a syringe (Hamilton, Reno, NV, USA). The HPLC column used was a reversed-phase C18 (Column 4.6 x 75 mm Phenomenex). Mobile phase A = water, B = methanol, Gradient at 0 min 90% B, at 15 min 100% B, at 20 min 100%, Column wash at 21 min 90% B, Flow rate 1.0 ml/min, UV detector-variable wavelength detector, 210 nm, standard cell, Column compartment temperature 20°C, Stop time 21 min, Post time 5 min, injection volume 5 ml. A Shimadzu MPS-2000 universal spectrophotometric scanner was used to determine the spectrograms of these four vitamins in n-butanol. The concentration of the compounds were quantified and the retention time, area and height of the compounds were also recorded.

## APPENDIX XIV

## ETHICAL APPROVAL LETTER FOR ANIMAL STUDY

**SRIMAD ANDAVAN ARTS & SCIENCE COLLEGE**
(Autonomous)
(Estd. 1996)
Managed By Sri Ranganatha Paduka Vidyalaya Trust (Regd.)
(Affiliated to Bharathidasan University)
Nationally Re-Accredited with 'A' Grade by NAAC
ISO 9001 : 2015 Certified Institution
No. 7, Nelson Road, Thiruvanai Kovil, Tiruchirappalli - 620 005.

To

J. Harine Sargunam (Roll No 16PHFNP005),
Research Scholar,
Avinashilingam Institute for Home Science and
Higher Education for Women,
Coimbatore - 641043

**28.09.2021**

**CERTIFICATE**

This is to certify that the research project entitled "**Effect of Aqueous Leaf Extract of Beta Vulgaris L on Estradial induced poly Cystic Ovary in Rats"** of the center **TanBio R & D Solutions,** Thanjavur as approved by the IAEC. The IAEC Approval number is **SAC/IAEC/BC/2020/C.P-004** dated **12.02.2020.**

Radhika 28/9/21
**Dr. J. RADHIKA**
Chairman – IAEC

CPCSEA-CHAIRMAN
790/PO/Re/S/03/CPCSEA
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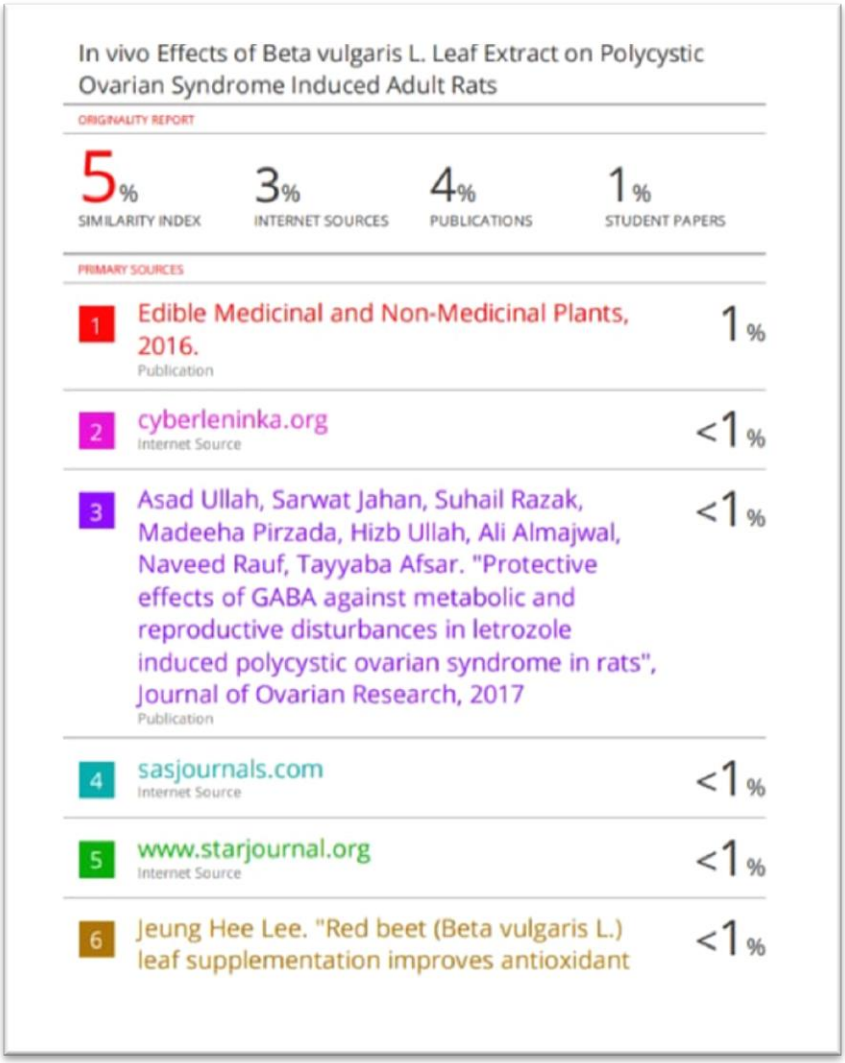
## APPENDIX XV

## PLAGIARISM REPORT (TURNITIN)



In vivo Effects of Beta vulgaris L. Leaf Extract on Polycystic Ovarian Syndrome Induced Adult Rats

ORIGINALITY REPORT

| 5% | 3% | 4% | 1% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Edible Medicinal and Non-Medicinal Plants, 2016.<br>Publication | 1% |
| 2 | cyberleninka.org<br>Internet Source | <1% |
| 3 | Asad Ullah, Sarwat Jahan, Suhail Razak, Madeeha Pirzada, Hizb Ullah, Ali Almajwal, Naveed Rauf, Tayyaba Afsar. "Protective effects of GABA against metabolic and reproductive disturbances in letrozole induced polycystic ovarian syndrome in rats", Journal of Ovarian Research, 2017<br>Publication | <1% |
| 4 | sasjournals.com<br>Internet Source | <1% |
| 5 | www.starjournal.org<br>Internet Source | <1% |
| 6 | Jeung Hee Lee. "Red beet (Beta vulgaris L.) leaf supplementation improves antioxidant | <1% |

# APPENDIX XVI

# DETAILS OF RESEARCH PUBLICATIONS

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University under Category A by MHRD, Estd. u/s 3 of UGC Act 1956)
Re-accredited with A+ Grade by NAAC, Recognised by UGC Under Section 12 B
Coimbatore - 641 043, Tamil Nadu, India

(Item No.5 of Check List)

Details of Research Publications

| S.No | Article | Journal | Other Details Vol./No./Page No./Year | Published in UGC-CARE/Scopus Indexed/Web of Science (*List of Journals in that category including the particular Journal to be attached) |
|---|---|---|---|---|
| 1. | Antioxidant activity of ethanolic leaves extracts of *Beta vulgaris* | Journal of Natural Remedies | ISSN : 2320-3358 (e) ISSN:0972-5547(p) Vol.21, No.8(1), Page No:216-221, 2020 | Scopus Indexed & Web of Science Indexed |
| 2 | GCMS Profile of Bioactive compounds with Therapeutic Potential in *Beta vulgaris* (L.) Ethanolic Leaf Extracts | Journal of Pharmaceutical Research International | ISSN: 2456-9119 Article No.JPRI.74089 Vol 33(43B), Page No:354-360, 2021 | Web of Science Indexed |

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar :

Supervisor :

Checked By:
HOD/Dean