Chapter 5

Assessment of qualitative and quantitative phytochemical properties of selected wild edible fruits

5.1 INTRODUCTION

Throughout the course of human history, various wild edible plants have been recognised for their remarkable nutritional and medicinal value across all geographical regions of the world. In addition to essential nutrients such as proteins, fats, carbohydrates, vitamins, and minerals, plant-based food contains an array of phytochemicals, including phenolic compounds alongside. These phytochemicals serve as potent antioxidants against reactive oxygen species (ROS) and provide a numerous potential health benefit. Extensive research has revealed the presence of numerous phytochemicals in plant-based foods, with some plant species containing over 100 different phytochemicals [1].

Fruits and vegetables generally serve as excellent sources of vitamins and minerals. Furthermore, they are abundant in bioactive compounds, predominantly polyphenols, which exert a wide range of health-promoting effects [2]. Fruits provide a sufficient level of nutrition for human beings, offering a rich source of vitamins and minerals, particularly vitamin C, as well as sugars, fiber, protein and energy [3]. They are characterized by their sweetness or sourness and consumption of fruits has been associated with a reduced risk of major chronic degenerative diseases [4]. The World Health Organization (WHO) recommends eating at least 400 g of fruits per day [5] for maintaining good health. Various researchers, such as Das et al., 2006 [6], Khan et al., 2011 [7] and Madhu et al., 2016 [8] have acknowledged the significance of the chemical constituents present in different plant species. While numerous studies have been conducted worldwide on the chemical constituents of valuable plants and fruits, limited research has been undertaken in the north-eastern region of India, particularly in the state of Manipur. Notable reports on phytochemical constituents from Manipur include the works of Sharma et al., 2013 [9], Devi et al., 2016 [10], and Nongmaithem, 2019 [11].

Furthermore, in recent times, particularly in the aftermath of the COVID-19 pandemic, there has been a notable upsurge in the daily diet of fruits and vegetables. This increased emphasis on incorporating these natural foods is a result of their recognized role in promoting overall health and immunity. Additionally, there has been a rise in the use of plant-based medicines or phytochemicals for the treatment of a wide range of health conditions and diseases, aligning with the trend towards seeking natural remedies and traditional therapeutic methods. Local practitioners in Manipur have extensively utilized wild edible plants to treat various ailments and diseases, including arthritis, stone problems, malaria, jaundice, and others since ancient times. Secondary metabolites, which have distinct characteristics and contribute to plants' pharmacological properties [12] are generally found in plants. Consequently, it is essential to investigate the phytochemical constituents of wild edible fruit plants of Manipur. Qualitative and quantitative phytochemical screening will provide valuable insights into the biochemical constituents present in these fruits, allowing for a comprehensive understanding of their characteristics.

Therefore, the primary objective of this study is to determine the phytochemical compositions, vitamin and mineral content, and the antioxidant properties of fifteen selected wild edible fruits from Manipur.

5.2 MATERIALS AND METHODS

The objective of the study was to comprehend the physical and chemical properties of the wild edible fruit species. Chemical analyses of the fruits were conducted to determine the nutritional value and potential applications of wild edible fruits. Levels of vitamins, minerals, and other nutrients were determined. Morphological characteristics of fruits such as fruits' size, shape, weight, colour, and other characteristics such as moisture content, pH, titratable acidity, solubility, and phytochemicals were recorded.

5.2.1 Experimental material

For the purpose of the study, fruits of fifteen species including *Antidesma bunius* (L.) Spreng, *Averrhoa carambola* L., *Dillenia indica* L., *Elaeocarpus floribundus* Bl., *Ficus cunia* Buch. -Ham.ex Roxb, *Garcinia pedunculata* Roxb., *Garcinia xanthochymus* Hook.f., *Microcos paniculata* L., *Phylanthus emblica* L., *Psidium guajava* L., *Rhus semialata* Murr., *Solanum betaceum* Cav., *Spondius pinata* (L.f.) Kurz, *Vangueria spinosa* (Roxb. ex Link) Roxb, and *Zizyphus mauritiana* Lamk. were selected. Fruits samples of wild edible fruit species which are mature and healthy were collected from different parts of study sites village areas and markets for determining various physical and chemical properties.

5.2.2 Sample collection and preparation

Mature, healthy, and disease-free fruits were collected from village market as well as from forest and nearby village areas of two Maring villages, Machi village (24°30'30.80" N; 094°08'29.96" E) and Laiching Minou village (24°30'28.14" N;

094°02'18.81" E) in Tengnoupal district. The fruits were also obtained from three main market areas, namely Pallel Bazar (24°27’03.55’’ N; 094°01’33.98’’ E), Kakching Bazar (24°29’53.95’’ N; 093°58’51.63’’ E), and Wangjing Bazar (24°35’53.89’’ N; 094°02’10.09’’ E) of Tengnoupal district, where the locals sold their collected fruits. The fruit plant species were carefully identified by referring to taxonomic characteristics of the species and other relevant taxonomic literature. The scientific nomenclature of the plant species was varified using The Plant List (www.theplantlist.org), and herbarium specimens were prepared for each species with a specified number (601-615), which are housed in the Tezpur University Herbarium House, Department of Environmental Science. The fruits were brought to the laboratory and washed thoroughly with running tap water and the pulp and seeds was separated manually. The fresh pulps were either used to generate fruit juice or dry samples. To obtain the fruit extract, 100g of fresh (wet) pulp was blended with 50 ml distilled water (taken in portion), and the pulp fibre was filter using a muslin cloth from the blended mixture. The process was repeated until 100 ml of fruit extract was obtained. The freshly extracted juice was then subjected to analysis. The fresh fruit pulp was also cut into pieces and oven-dried at temperatures ranging from 40^0C to 50^0C until a constant weight was achieved. Further, the dried fruit samples were ground and then stored in an airtight container with a proper identification level for further chemical analysis.

5.2.3 Physical properties of fruits

Physical characteristics such as weight, circumference, length, and shape of the fifteen wild edible fruits were measured using appropriate tools such as weighing machine, ruler, graph paper, pencil, and rope. 100 fruits of smaller fruits namely, *Antidesmus bunius, Phyllanthus emblica, Rhus semialata,* and *Ziziphus mauritiana* were considered, while 50 fruits were used to estimate the weight of the remaining fruit samples.

5.2.4 Preparation of plant extract

The subsequent extraction of the sample was carried out using aqueous and ethanol as solvent for qualitative and quantitative analysis [13].

5.2.4.1 Preparation of aqueous extract

A quantity of 5 grams of each oven-dried powdered sample was mixed with 25 millilitres of deionized water, followed by boiling at a temperature range of 50-60°C for a duration of 30 minutes on a water bath. Subsequently, the resultant mixture was filtered through Whatman No. 1 filter paper to obtain the filtered extract of the plant samples, which was then utilized for subsequent phytochemical analysis.

5.2.4.2 Preparation of solvent extract

In a conical flask, 10 grams of oven-dried fruit powder was mixed with 100 millilitres of organic solvent, specifically 70% ethanol. The flask was subsequently sealed with cotton wool and placed on a rotary shaker, which was operated at a speed of 190-220 rpm for a duration of 24 hours. After completion of the extraction, the supernatant was carefully collected, and the solvent was removed through evaporation until the volume was reduced to one-fourth of the original. The resultant solution was then transferred and stored in an air-tight container at a temperature of 4°C until further phytochemical analysis.

5.2.5 Physico-chemical properties

Various standard methods were employed to determine the physico-chemical parameters, including moisture content (MC), total solids (TS), solubility (S), pH, titratable acidity (TA), and colour index. The analysis was carried out in triplicate.

5.2.5.1 Moisture content (MC) and total solids (TS)

The oven drying method was employed following Method 977.11 [14] to determine the moisture content. The method involves drying the fruit sample under controlled temperature until a constant weight is obtained. For this purpose, 100g of the sample was accurately weighed and dried in an oven at 65 to 70^0C until a constant weight was achieved. The estimation was carried out in triplicate, and the moisture content was calculated using Eq. (5.1) and expressed in percentage (%).

$$\text{Moisture content } (\%) = \frac{(W_1 - W_2)}{W_1} \times 100 \tag{5.1}$$

where, W_1 and W_2 represent the weight of the sample before drying and after drying, respectively.

The total solids (TS) content is an indication of the material remaining after all the water has been evaporated. Thus, the total solids were determined using Eq. (5.2) and expressed as a percentage.

$$\text{Total solids } (\%) = (100 - \%\ \text{Moisture}) \tag{5.2}$$

5.2.5.2 Solubility (S)

The method described by Chau et al., 2007 [15] was used to determine the solubility of the fruit sample. In brief, samples were mixed with distilled water at a ratio of 1:10 (w/v), stirred for 1 hour at room temperature, and then centrifuged at 1500 rpm for 10 minutes. The supernatant was collected, dried, and weighed. The percentage of solubility was calculated using the following Eq. (5.3):

$$\text{Solubility } (\%) = \frac{W_f}{S} \times 100 \tag{5.3}$$

Here, W_f represents the final weight (in grams) of supernatant after drying and S represents the weight (in grams) of the sample taken.

5.2.5.3 pH and titratable acidity (TA)

To determine the pH, a pH meter (pH 700, Eutech Instrument, Singapore) was used to measure the H^+ ion activity, which indicates active acidity. The pH of the fresh juice and extract from dry samples were measured. For the dry samples, 1.25 grams of the dried test sample were weighed and transferred into a conical flask. Then, 20 ml of standard phosphate buffer solution (pH 7.0) was added and shaken for 1 minute. After one hour, the solutions were filtered through filter paper, and the pH value was measured.

To determine the titratable acidity, the total titratable acidity was determined by the titration method. 1 gram of the dried and powdered sample was dissolved in 10 ml of distilled water, and 2 to 3 drops of 1% phenolphthalein indicator was added. The solution was titrated against 0.1 N sodium hydroxide. The percentage of titratable acidity was calculated using Eq. (5.4) and expressed as citric acid equivalent (% w/w).

$$\text{Titratable acidity}(\%) = \frac{\text{Titre value} \times \text{Normality NaOH} \times \text{Equivalent wt.of citric acid} \times 100}{\text{Sample volume taken} \times \text{sample weight} \times 1000}$$

$$\text{Titratable acidity}(\%) = \frac{\text{Titre value} \times 0.1 \times 64 \times 100}{10 \times 1 \times 1000} \qquad (5.4)$$

5.2.6 Colour measurement

Colour analysis is a crucial component of food science because it is commonly used to evaluate the quality of food products. A Colorimeter (Ultrascan VIS, Hunterlab, USA) was used to analyse the colour of dried fruit samples. The colorimeter produced CIELab coordinates (a^*, b^*, L^*, L, b, and a) that provide a three-dimensional representation of a sample's colour based on human eye perception. L^*

is an indicator of lightness, while a^* and b^* are chromaticity coordinates that represent green/red and blue/yellow, respectively. L^* is an approximate measure of luminosity, which determines the extent to which each colour can be considered equivalent to a member of the grey scale between black and white, with values ranging from 0 to 100. a^* assumes positive values for reddish colours and negative values for greenish colours, while $b*$ assumes positive values for yellowish colours and negative values for bluish colours [16].

In addition to the CIELab coordinates, derived colour indices were also calculated to further characterize the colour of the dried samples. These colour indices included the whiteness index (WI), which is a measure of how white a sample appears, the yellowness index (YI), which is a measure of the yellow colour of a sample, and the browning index (BI), which is a measure of the degree of browning in a sample.

The equations used to calculate these colour indices were proposed by previous studies [16–18], and in the present study, they were referred to as Eqs. (5.5) – (5.7). The results of the colour analysis provide valuable information about the dried samples quality and can be used to assess their suitability for various food applications.

$$WI = L - 3b + 3a \tag{5.5}$$

$$YI = \frac{142.86\, b^*}{L^*} \tag{5.6}$$

$$BI = 100 \times \left(\frac{X - 0.31}{0.17} \right)$$

$$\text{where, } X = \frac{(a + 1.75L)}{(5.645\, L + a - 3.012\, b)} \tag{5.7}$$

5.2.7 Compositional analysis

5.2.7.1 Qualitative analysis

To determine the presence of phytochemical components in the plant extracts, standard methods described by Harborne, 1998; Sofowora, 1993; Kokate et al., 2002; and Evans, 2002 [19–22] were used. These standard protocols were used to check the presence of proteins, carbohydrates, tannins, phenols, flavonoids, saponins, alkaloids, terpenoids, steroids, cardiac glycosides, phlobatannins, anthocyanins, and quinones in the fruit samples.

5.2.7.2 Reagents/Chemicals

Reagents required to conduct the screening of phytochemical components includes Million's reagent, ninhydrin, ammonium hydroxide, copper sulphate, sodium hydroxide, concentrated sulphuric acid, Fehling's reagent, Benedict's reagent, Molisch's reagent (which contains 1-Naphthol in alcohol or chloroform), ferric/iron chloride, lead acetate, Mayer's reagent (which contains Mercuric chloride and potassium iodide), Wagner's reagent (which contains iodine and potassium iodide), hydrochloric acid, and ammonia.

5.2.7.3 Preparation of Reagents

1. Molisch's reagent: 15g of 1-Naphthol was dissolved in 100ml of either alcohol or chloroform.

2. Mayer's reagent: 1.3g of mercuric chloride and 5g of potassium iodide were separately dissolved in distilled water, mixed both the solutions, and made up to 100ml with distilled water.

3. Wagner's reagent: 2g of iodine and 6g of potassium iodide were dissolved in 100ml of distilled water.

4. Fehling's reagent: Fehling's A and B solution were mixed in a 1:1 ratio.

5.2.8 Steps involved in qualitative analysis of plant extracts.

5.2.8.1 Test for Proteins:

a) Million's Test: 3ml of plant extract was mixed with 2ml of million's reagent. The formation of a white precipitate which turns red upon gentle heating indicates the presence of proteins.
b) Ninhydrin Test: When a few drops of 0.2% w/v Ninhydrin solution was added in 2 ml of plant extract, and if violet colour forms then it indicates the presence of amino acids and proteins.
c) Xanthoproteic Test: 1ml of concentrated sulphuric acid was added to 1ml of extract. The formation of a white precipitate that turns yellow on boiling and orange on addition of ammonium hydroxide (1ml) indicates the presence of proteins containing tyrosine and tryptophan.
d) Copper sulphate Test: 1ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added to 2ml of plant extract. The formation of a violet colour indicates the presence of peptide linkage molecules in the sample extract.

5.2.8.2 Test for Carbohydrates

a) Molisch's Test: 1ml plant extract was mixed with a few drops of Molisch's reagent, and then 1ml of concentrated sulphuric acid was carefully added to the side of the tubes. After allowing the mixture to stand for 2 to 3 minutes, the appearance of a red or dull violet colour indicated the presence of carbohydrates in the sample.
b) Fehling's Test: To 2ml of extracts, 2ml of Fehling's reagent was added and heated in boiling water bath for 10 minutes. The appearance of a yellow and then brick-red precipitate indicates the presence of reducing sugar.
c) Benedict's Test**:** To 2ml of plant extract, 2ml of Benedict's reagent was added and heated in boiling water bath for 10 minutes. Changes in colour (yellow, green, or red) indicates the presence of reducing sugars.

5.2.8.3 Test for Tannins:

a) Alcoholic Ferric Chloride Test: 2ml of 10% w/v alcoholic ferric chloride solution was added to 2ml of plant extract. The formation of a brownish blue or black colour indicates the presence of tannins.

5.2.8.4 Test for Phenols:

a) Aquatic Ferric Chloride Test: To 2ml of plant extract, 2ml of 5% w/v aqueous ferric chloride solution was added, formation of a blue colour indicates the presence of phenols.

5.2.8.5 Test for Flavonoids:

a) Alkaline Reagent test: When 2 ml of plant extract was mixed with 2ml of 10% NaOH solution and shaken vigorously. The appearance of intense yellow colouration that turns colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.
b) Lead Acetate Test: 1ml of 10% lead acetate solution was added to 1ml of plant extract. The appearance of a yellow colour indicates the presence of flavonoids.

5.2.8.6 Test for Saponins:

a) Foam Test: To 2ml of extract, 5ml of distilled water was added and shaken vigorously. The formation of persistent foam or froth indicates the presence of saponins.

5.2.8.7 Test for Alkaloids:

a) Mayer's Test: To 3ml of plant extract, 1ml of Mayer's reagent was added. The appearance of a white precipitate indicates the presence of alkaloids.

b) Wagner's Test: To 3ml of plant extract, 1ml of Wagner's reagent was added. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

5.2.8.8 Test for Terpenoids and Steroids:

a) Salkowski Test: 5ml of plant extract was mixed with 2ml of chloroform ($CHCl_3$) and shaken thoroughly. Then, 3ml of concentrated sulphuric acid was carefully added to the mixture at an inclined angle. The appearance of reddish-brown coloration indicates the presence of terpenoids, while the appearance of red coloration indicates the presence of steroids.

5.2.8.9 Test for Cardiac glycosides:

a) Keller-Killiani Test: 5ml of plant extract were mixed with 2ml of glacial acetic acid and a few drops of 5% w/v ferric chloride solution. To this mixture, 1ml of concentrated sulphuric acid was added slowly at an inclined position. The formation of brown ring at the interference indicates a deoxy sugar, which is characteristic of cardenolides.

5.2.8.10 Test for Phlobatannins:

a) Hydrochloric acid Test**:** 2ml of hydrochloric acid was added in 2ml of plant extract and heated. The formation of a red precipitate indicates the presence of phlobatannins.

5.2.8.11 Test for Anthocyanins:

a) Hydrochloric acid Test: To 2ml of plant extracts, 2ml of 2N hydrochloric acid and 2-3ml of 10% ammonia solution were added. The appearance of a pinkish or bluish violet colouration indicates the presence of anthocyanins.

5.2.8.12 Test for Quinones:

a) Borntrager's Test: To 2ml of plant extract, 2ml of concentrated sulphuric acid were added. The formation of a red colour indicates the presence of quinones.

5.2.9 Quantitative analysis of proximate composition

In the proximate composition, parameters such as ash content, fat content, calorific value, total carbohydrate contents, and total protein contents were estimated using standard protocols. All the experiment was conducted in triplicate to ensure the reliability of the results.

5.2.9.1 Determination of ash content

Ash content represents the inorganic residue (minerals) remaining after ignition and complete oxidation of organic matter. To determine ash content, plant sample is heated at 500-600°C in a muffle furnace through dry ashing. The procedure involves placing 1g of the dried sample in a clean and pre-weighed porcelain crucible, heating it for about 5-6 hours in a muffle furnace, and then cooling it down to room temperature using a close desiccator. The crucible with the resulting greyish-white ash is then weighed to obtain the final weight. The percentage of ash content is determined using the following Eq. (5.8) as suggested [23]:

$$\% \text{ Ash content} = \left(\frac{W_2 - W_0}{W_1} \right) \times 100 \tag{5.8}$$

Here, W_0 is the weight of crucible, W_1 is the weight of the sample, and W_2 is the weight of crucible and ash (final weight).

5.2.9.2 Estimation of crude fat

The Association of Official Analytical Chemists (AOAC) Method No. 920-39 [24] was used to determine total crude fat content using petroleum benzene as a solvent through the Soxhlet method, which is a typical practice for crude fat analysis. The following steps were followed:

- 5 grams of sample were accurately weighed into a thimble/flask and dried in an oven at 102ºC for 5 hrs.

- The thimble containing the dried sample was inserted into a Soxhlet liquid/solid extractor.

- A clean, dry 150 ml round bottom flask was accurately weighed, and 90 ml of petroleum benzene was added to it. The extraction unit was then assembled over an electric heating mantle.

- The solvent was heated in the flask until it boiled, and the extraction process continued for approximately 6 hours, with the solvent dripping from the condenser into the sample chamber at a rate of around 6 drops per second.

- The extraction unit was then removed from the heat source, and the extractor and condenser were detached. The flask on the heat source was replaced, and the solvent was evaporated off. The flask was then placed in an oven at 60 to 80ºC and dried until a constant weight was obtained (for 1 to 2 hours).

- The flask was then placed in a desiccator to cool, and the weight of the flask with its contents was measured. This process was repeated three times for each sample.

- The percentage of the crude fat of the samples were calculated using the following Eq. (5.9), provided below:

$$\% \text{ Crude fat} = \left(W_2 - W_1\right) \times \frac{100}{S} \quad (5.9)$$

Here, W_1 is the weight of the empty flask, W_2 is the weight of the flask and extracted fat, and S is the weight of sample.

5.2.9.3 Calorific value

The auto bomb calorimeter (Changsha Kaiyuan Instrument Co. Ltd; Model: 5E-1AC/ML) was used to measure the calorific value of the dried sample [25]. The principle of the auto bomb calorimeter involves determining the energy value of solid and non-volatile fuels. In this estimation, the dried fruit sample was used as the fuel, with carbohydrates, proteins, and fats serving as the fuel components. The experiment involved burning a unit mass of the fuel and allowing the heat generated to be absorbed in water, where it was recorded as a function of temperature.

5.2.9.4 Total Carbohydrate

The total carbohydrate content was estimated using the phenol-sulfuric acid method [23]. This colorimetric method is based on the reaction of hot acidic medium with glucose present in the sample, which is dehydrated to hydroxymethyl furfural. The resulting product forms a green coloured complex with phenol that has an absorption maximum at 490 nm. While this method detects virtually all classes of carbohydrates (mono-, di-, oligo-, and polysaccharides) however, the absorptivity of the different carbohydrates varies.

The reagents required for the total carbohydrate content estimation are 2.5N HCl, sodium carbonate, 5% phenol, 96% reagent grade sulfuric acid, and a standard glucose solution. For preparing the standard glucose solution, 100 mg of glucose was dissolved in 100 ml of distilled water to obtain a stock solution. From this, a working solution was prepared by diluting 10 ml of the stock solution with 90 ml of distilled water, resulting in a concentration of 0.1 mg/ml. The following steps were followed:

- The sample (100mg) was hydrolysed with 5ml of 2.5N HCl for 3 hours in a boiling water bath and then cool down to room temperature.

- Neutralised the solution with sodium carbonate until the effervescence ceases and made up the volume to 100ml with distilled water and then centrifuged.

- After centrifuging, 0.2 ml of the sample solution was taken, made up to 1 ml with distilled water.

- For obtaining the standard curve for carbohydrate, varying volumes of the working solution of standard glucose (0.2, 0.4, 0.6, 0.8, 1 ml) in a series of test tubes. 1ml of distilled water was set as blank.

- 1 ml of phenol solution and 5ml of 96% H_2SO_4 were added to each test tube, which was shaken well and rested for 10 minutes.

- The test tubes were then placed in a water bath at 25-30°C for 20 minutes before measuring the absorbance at 490nm.

- Then, by using the standard graph, the total amount of total carbohydrate present in the sample was calculated.

- The percentage of the total carbohydrate of the samples were calculated using the following Eq. (5.10), provided below:

$$\%\,\text{Carbohydrate} = \frac{\text{sample concentration} \times \text{dilution factor}}{\text{sample mass}} \times 100 \qquad (5.10)$$

Where, dilution factor is amount of reagent added divided by amount extract used.

5.2.9.5 Total protein

Lowry's Method [26] was employed to estimate the total protein. This method is widely used due to its sensitivity in producing a consistent value. The method is based on the principle that the phenolic groups of tyrosine and tryptophan residues in a protein will react with Folin-Ciocalteau reagent to form a blue-purple coloured complex that absorbs light at 660 nm wavelength. The intensity of the colour depends on the amount of these aromatic amino acids in the protein, which varies across different proteins. Bovine Serum Albumin (BSA) is commonly used as a standard protein because it is cost-effective, highly pure, and readily available.

To perform the estimation, the following reagents are required:

1. Reagent A: 2% sodium carbonate in 0.1N sodium hydroxide

2. Reagent B: 0.5% copper sulphate in 1% potassium sodium tartrate

3. Reagent C: An alkaline copper solution, prepared by mixing 50 mL of Reagent A with 1 mL of Reagent B prior to use.

4. Reagent D: Folin-Ciocalteau reagent

5. BSA (standard) solution:

   Stock solution: 50mg of BSA dissolved in 50 ml of distilled water.

   Working solution: Diluted 10 ml of stock solution with 50 ml of distilled water in a standard flask). 1ml of this working solution contains 0.2 mg protein.

To carry out the procedure, 0.2, 0.4, 0.6, 0.8, and 1 mL of the BSA working solution were pipetted into a series of test tubes. Additionally, 0.2 mL of the sample extract was pipetted into other test tubes and made up to 1 mL with distilled water. A test

tube with 1 mL of distilled water serves as the blank. To all the test tubes, including the blank, 5 mL of Reagent C was added and mixed well. The mixture was allowed to stand for 10 minutes before adding 0.5 mL of Reagent D, which was then mixed well. The test tubes were incubated at room temperature in the dark for 30 minutes to allow the blue colour to develop. The absorbance reading was taken at 660 nm, and the total amount of protein was calculated using the standard curve and expressed in ppm or mg/g or g/100g.

5.2.10 Phytochemicals

In phytochemical estimation, the principal secondary metabolites such as total phenol, total flavonoid, and total tannin were determined.

5.2.10.1 Total phenolic content (TPC)

The Folin-Ciocalteu method was used for the estimation of total phenol content [27,28]. In this method, the phenols in the sample react with phosphomolybdic acid in the Folin-Ciocalteu reagent under alkaline conditions, resulting in the formation of a blue-coloured complex called molybdenum blue.

The following reagents are required for the estimation:

1. 80% ethanol (reagent grade)

2. Folin’s reagent

3. 20 % Sodium carbonate

4. Standard solution:

   Stock solution: 100 mg Gallic acid in 100 ml water

Working solution: 10 times dilution of standard solution i.e., 10ml of the stock solution was diluted to 100ml with distilled water (concentration of the stock solution is 0.1mg/ml)

5g of the sample was weighed, ground with a pestle and mortar in ten times volume of 80% ethanol and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected, and the residue was re-extracted with five times the volume of 80% ethanol, and the supernatant was pooled. The supernatant was then evaporated to dryness, and the residue was dissolved in a known volume of distilled water (5ml). A series of test tubes were prepared by pipetting out 1 ml of the aliquot/extract and gallic acid (0.1, 0.2, 0.4, 0.6, 0.8, and 1mg/ml), with 1 ml of water serving as the blank. The volume was made up to 5 ml with water, and 0.5 ml of Folin's reagent was added to all the tubes, including the blank, and shaken well. After 5 minutes, 2 ml of 20% sodium carbonate was added to all the tubes, followed by thorough mixing. The mixture was then incubated for 2 hours at room temperature, and the absorbance was measured at 750 nm. This process was repeated three times for each sample, and gallic acid was used as the standard. A calibration curve was plotted to calculate the amount of total phenol content in the samples, expressed in mg of gallic acid equivalent per gram (mg GAE/g) of dry mass.

5.2.10.2 Total flavonoid content (TFC)

The total flavonoid content was estimated using an aluminium chloride colorimetric assay [29,30]. The procedure involved adding 1 ml of solvent extract/aliquots and 1 ml of quercetin solution, while varying concentrations of quercetin (0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/ml) were placed into a series of test tubes. To this, 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution are added. After 5 minutes, 0.3 ml of 10% aluminium chloride was added, and the mixture was incubated for 6 minutes. Then, 2 ml of 1M sodium hydroxide was added, and the volume was adjusted to 10 ml with distilled water and mixed well. The resultant orange-yellow colour was measured for absorbance at 510 nm using a UV-visible spectrophotometer (Jasco V-630). The blank was prepared using distilled water, and quercetin was used as the

standard. The samples were tested in triplicates, and the concentration of total flavonoids was calculated in mg of quercetin equivalent (QE)/g of dry mass, using a calibration curve.

5.2.10.3 Total tannin content (TTC)

The tannin content was analysed using a modified version of the Folin and Ciocalteu's method [31]. The procedure involved taking 1ml of sample extract and adding 3.0 ml of distilled water, 0.5 ml of Folin Ciocalteu's reagent, and 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 1mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg TAE/g of extract.

5.2.11 Antioxidant activity

There are several methods available for determining antioxidant activity, including commonly used methods such as DPPH, ABTS, and FRAP assays [32]. However, the effectiveness of antioxidant compounds can be influenced by various factors, including laboratory conditions [33]. Application of free radical diphenylpicrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) are widely accepted methods for evaluate antioxidant activity. In this study, a modified version of the DPPH* scavenging method was used [34], along with FRAP assay [35] and in-vitro antioxidant activity [36] were executed to determine the reducing power.

5.2.11.1 Extraction of plant extract

Extraction is the crucial step to extract the desired chemical components from the plant materials. To extract the compounds for this analysis, 5g of powdered sample was added to a conical flask (150 ml) and mixed with 50 ml of methanol (1:10; w/v). The mixture was placed in an orbital shaker for 24 hours at 160 rpm, and then transferred to a 50 ml centrifuge tube and centrifuged at 10000 rpm for 10 minutes after cooling to room temperature. The supernatant was filtered using Whatman No.

1 paper and collected in a beaker. The pellet was returned to the flask and the process was repeated for 2 hours in the shaker. All the supernatant was combined and evaporated in a hot-air oven at 40-50^{0}C overnight. The resulting dried extract was collected using a spatula and transferred into a glass vial, then dissolved in methanol before the assay [37].

5.2.11.2 DPPH Radical Scavenging Activity

The DPPH method is a commonly used antioxidant assay that measures the ability of a substance to scavenge free radicals. The free radical used in this assay is 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is a stable free radical that is purple in colour. When an antioxidant molecule is added to the DPPH solution, it reduces the DPPH radical, resulting in a colour change from purple to yellow or colourless.

To conduct the assay, the following reagents are required:

1. 100 µM DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate)

2. 0.1 mg per ml ascorbic acid (as positive control)

3. Methanol

4. DPPH solution: Preparing a stock solution of DPPH (608.6 µM) involves dissolving 24 mg (0.024g) of DPPH in 100 ml of methanol. The stock solution is then diluted (six times) to a working solution, with the absorbance adjusted to around 1.0 at 517 nm using stock solution or methanol.

5. Positive control solution: A positive control, such as ascorbic acid, was also prepared. The preparation of ascorbic acid stock solution involves dissolving 100 mg (0.1g) of ascorbic acid in 100 ml methanol. Then the stock solution is diluted (ten times) to get the working solution.

To perform the assay, different concentrations such as 20, 40, 60, 80, and 100 µl of extract/standard solution were taken to separate test tubes and made up to 100 µl with methanol. The solution was then added with 1900 µl of DPPH working solution and incubated for a specific amount of time, typically 30 minutes, at room temperature. The absorbance was measured at 517 nm, and the percentage of scavenging effect was calculated using the Eq. (5.11):

$$\% \text{ scavanging effect} = \left(\frac{A_0 - A_1}{A_0} \times 100 \right) \tag{5.11}$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the test sample. The IC_{50} value, which is the concentration of the test substance that causes a 50% reduction in the DPPH radical, can also be calculated using the data obtained from the assay, which is by plotting a graph between the % inhibition (scavenging effect) and the concentration of the sample.

5.2.11.3 FRAP antioxidant assay

The FRAP (Ferric reducing antioxidant power) assay is a commonly used method that employs antioxidants as reductants in a redox-linked colorimetric reaction. In this assay, Fe^{3+} (ferric) is reduced to Fe^{2+} (ferrous) via the use of a low pH environment, resulting in the formation of a coloured ferrous-probe complex from a previously colourless ferric-probe complex [35].

To conduct the FRAP assay, the following reagents are needed:

1. 0.3M acetic acid
2. 0.3M sodium acetate
3. 40mM hydrochloric acid
4. 10mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ)

5. 20mM Ferric chloride hexahydrate ($FeCl_3.6H_2O$)

6. Ascorbic acid

To prepare the necessary reagents, the following steps must be taken:

1. 0.3M acetate buffer pH 3.6: 463ml of 0.3M acetic acid and 37 ml of 0.3M sodium acetate were mixed properly and adjusted the pH to 3.6 using 1M NaOH or 1M HCl.

2. Standard solution preparation (Ascorbic acid): 100 mg (0.1g) of ascorbic acid was dissolved in 100 ml methanol to make the stock solution and it was diluted ten times to get the working solution.

3. Working FRAP reagent: 100 ml of 0.3M acetate buffer pH 3.6, 10 ml of 10mM TPTZ, and 10 ml of 20mM ferric chloride hexahydrate were mixed properly and warmed at 37°C prior to use for 30 minutes.

To perform the assay, 100µl of diluted sample were pipetted out into test tubes. For the standard antioxidant, pipetted out 20, 40, 60, 80, and 100µl of ascorbic acid working solution into different test tubes and made up to 100µl using methanol. In addition, 100µl of solvent used in the sample preparation (methanol) was served as blank. Then, 1.9 ml of freshly prepared FRAP reagent was added in all the test tubes and incubated for 5 minutes in room temperature after properly mixed. The absorbance was then measured at 593 nm using a spectrophotometer. The antioxidant activity of the standard curve was plotted and calculated for the samples accordingly. The activity was expressed as mg ascorbic acid equivalent/g sample.

5.2.11.4 In-vitro antioxidant activity assay

The ferric reducing antioxidant power method was used to assess the in-vitro antioxidant activity. 2.5 ml of extract was mixed with 1 ml of 0.2 M phosphate buffer pH 6.6 and 1 ml of 1% potassium ferricyanide, and the resulting mixture was

incubated in a water bath at 50°C for 20 minutes. The reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid and then centrifuged for 10 minutes. A 2.5 ml aliquot was taken and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride solution, resulting in a green colour change. After 10 minutes, the absorbance was measured at 593 nm using a spectrophotometer. Reagent and solvent blanks were used, and ascorbic acid (0 to 1 mg/ml) served as the standard. The in-vitro antioxidant activity was expressed in ascorbic acid equivalent (AAE) mg/g sample.

5.2.12 Vitamins

5.2.12.1 Vitamin C

The concentration of vitamin C in the fresh sample was determined using the titration method [38], while for dry sample titrimetric method described by Baraket et al. in 1973 [39] was used .

For the titration, 0.005mol/L iodine or potassium iodate solution, 1% starch indicator solution, distilled water, and 20% copper sulphate solution were needed. To prepare the 1% starch indicator solution, 1g of starch was dissolved in 10ml of distilled water and added to boiling distilled water (90 ml), then cooled to room temperature. To prepare the 0.005mol/L iodine solution, 2g of potassium iodide and 1.25g of iodine were weighed into a 100 ml beaker. A few ml from measured 1L distilled water was added and swirled for a few minutes until iodine is dissolved. Then the iodine solution was transferred to a 1L volumetric flask and made the solution up to 1L with distilled water.

To prepare the sample for the analysis, the following steps were undertaken:

1. The fresh sample was prepared by grinding 100g of the sample with distilled water added in 10 ml portions several times. The resulting pulp was then

strained through cheesecloth, and the filtrate was collected in a 100 ml volumetric flask until it reached the 100 ml mark.

2. To prepare a dry sample, 5 grams of the sample were measured and placed into an extraction tube. Then, 100 millilitres of an EDTA/TCA (2:1) extracting solution were added and shaken for 30 minutes. The mixture was then placed to a centrifuge tube and spun at 3000 rpm for approximately 20 minutes. After centrifugation, the supernatant was then transferred to a 100 ml volumetric flask and filled up to the 100 ml mark using the extracting solution.

For the fresh sample titration, 10ml of the sample extract was mixed with 75 ml of distilled water and 1 ml of starch indicator solution in a conical flask, then titrated with 0.005 mol/L iodine solution until a dark-black colour due to the starch-iodine complex was observed. For the dry sample titration, 20 ml of the extract was mixed with a few drops of 1% starch indicator and titrated against 20% $CuSO_4$ to get a dark end point. The process was repeated three times for all the samples. 1ml of iodine solution (0.005mol/L)/ copper sulphate solution is equivalent to 0.88 mg of ascorbic acid. Hence the concentration of the unknown sample can be derived from the amount of iodine solution used during the titration and expressed as mg/100g fresh weight or dry weight.

5.2.12.2 Vitamin B_1 (Thiamine) and vitamin B_2 (Riboflavin)

Thiamine (Vitamin B1) and riboflavin (Vitamin B2) were quantified using colorimetric analysis [40], and the results were expressed in mg/100g. The following reagents were required: dry sample, ethanolic sodium hydroxide, acid alcohol, standard potassium dichromate, 20% alcohol, 50% ethyl alcohol, 5% potassium permanganate, 30% H_2O_2, 40% sodium sulphate, 0.1 N sodium hydroxide, and standard (thiamine and riboflavin)

Reagent preparation:

1. Ethanolic sodium hydroxide: 2.1 gram of sodium hydroxide was dissolved in 5 ml of distilled water and added sufficient amount of aldehyde-free ethanol to produce 500 ml. The solution was allowed to stand in a tight stopper bottle for 24 hours. Then quickly decant the clear supernatant liquid into a suitable, tightly closed container.

2. Acid alcohol: 2.0 ml of concentrated hydrochloric acid and 98 ml of 95% ethyl alcohol were mixed properly.

3. Standard potassium dichromate: 2.45g of reagent grade potassium dichromate to a clean dry weighing bottle. Using a beaker and watch glass arrangement, dried the solid in an oven at 110^{o}C for one hour. The potassium dichromate was cooled down in its weighing bottle for 30 minutes in a desiccator. The weight of the weighing bottle and its contents was obtained to the nearest milligram, then transferred the potassium dichromium to a clean beaker, and reweighed the empty weighing bottle to the nearest milligram to determine the weight of the potassium dichromate by difference. The dried potassium dichromate was dissolved by adding a small amount of distilled water to the breaker. This solution was transferred to a clean 500 ml volumetric flask and brought the solution up to the calibration mark with distilled water and mixed them thoroughly.

4. Thiamine stock solution: 10 mg of the thiamine is dissolved in 20% alcohol and then diluted with 100 ml additional acidified alcohol (100 ppm). Different concentrations of working solution were prepared (10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm) by diluting the sock solution using d.H_2O.

5. 50% ethanol: 53 ml of the 95% ethanol were taken in a 100 ml volumetric flask and made its volume up to 100 ml with d.H_2O.

6. 5% potassium permanganate: 5 grams of potassium permanganate was dissolved in 100 ml d.H_2O.

7. 40% sodium sulphate: 40 grams of sodium sulphate was dissolved in 100 ml d.H_2O.

8. Riboflavin stock solution: It was prepared by dissolving 100 mg of riboflavin in 100 ml of 0.1 N sodium hydroxide which gives concentration of 1000 ppm. 10 ml of stock solution was taken and diluted up to 100 ml using 0.1 N sodium hydroxide to produce a concentration of 100 ppm which was used in the solution preparation for the standard calibration curve (10, 15, 20, 25, and 30 ppm).

To prepare the sample for the analysis, the following steps were considered:

1. For the determination of thiamine: 5 grams of dry sample were homogenised with 50 ml of ethanolic sodium hydroxide. It was filtered into a 100 ml volumetric flask.

2. For the determination of riboflavin: 5 grams of dry sample was extracted with 100 ml of 50% ethanol and shaken for 1 hr. This was filtered into a 100 ml flask using Whatman No. 1.

To conduct the experiment, the following steps were involved:

1. For the determination of thiamine: 10 ml of filtrate or different concentration of standard solutions were mixed with 10 ml of potassium dichromate. The absorbance was then read at 507 nm and a calibration curve was plotted using different concentrations of standard solutions. Originally, the wavelength for reading absorbance was 360 nm but it was modified to 507 nm as suggested by Al-Ward and Hussein [41]. A blank sample was prepared with d.H_2O.

2. For the determination of riboflavin: 10 ml of the extract was mixed with 10 ml of 5% potassium permanganate in a 50 ml volumetric flask. To this solution, 10 ml of 30% H_2O_2 was added slowly from sides and then allowed to stand over a hot water bath for about 30 minutes. Then, 2 ml of 40% sodium sulphate was added and the volume was made up to 50 ml with d.H_2O. The absorbance was read at 450 nm.

The concentration of thiamine and riboflavin in the samples was reported as mg/100g dry weight, and the analysis was repeated three times for all samples.

5.2.13 Mineral elements

Eight mineral elements were evaluated, comprising of four macro-elements namely sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca), and four micro-elements, namely iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn). The instrument ICP-MS (Thermofisher Scientific, iCAP RQ) was used to detect minerals such as Na, K, Mg, Fe, Mn, Cu, and Zn, while Ca was estimated using the flame photometry method.

To extract elements, a PTFE (polytetrafluoroethylene) vessel containing 0.5 g of moisture-free powdered samples was used. Along with the sample, 9 ml of nitric acid (HNO_3), 0.5 ml of hydrochloric acid (HCl), and 1 ml of hydrogen peroxide (H_2O_2) were added. This mixture was then subjected to double acid digestion using a microwave digestion system (Anton Paar, Multiwave Go) at a temperature of 180^{o}C, pressure of 35 bar, ramp time of 5 minutes, and holding time of 20 minutes [42].

5.2.13.1 Element analysis using ICP-MS (Inductively coupled plasma mass spectrometry)

The ICP-MS technique (Thermofisher Scientific, iCAP RQ model) was employed for the quantification of chemical elements. The double acid digested samples were diluted 50 times with double-distilled water and filtered using a 0.25 mm syringe

filter. Standard solutions with varying concentrations (in ppb) of the desired elements were prepared to calibrate the system for all the elements of interest. The concentration of the elements was reported in mg/g. The operational conditions of the ICP-MS are summarized in Table 5.1.

Table 5.1 Instrumental analytical conditions for the ICP-MS of element analysis

Parameters	Settings
Vacuum	3×10^{-6}
Argon gas (Psi)	130-150
Peristatic pump pressure (rpm)	40
Spray chamber temp. (°C)	2.75
Cool gas flow (l/min)	13
Auxiliary flow (l/min)	0.8
Pressure of nebulization (bar)	>1
Plasma view	Axial
Uptake time (s)	30
Stabilization time (s)	5

5.2.13.2 Estimation of calcium using flame-photometer

Flame atomic emission spectrometry, commonly known as flame photometry, is a rapid, simple, and highly sensitive technique for detecting specific trace metal ions such as Na, K, Li, and Ca that can be easily excited and do not require extremely high temperatures. To quantify calcium, the 50-fold diluted samples were analysed using the Systronics Flame Photometer 128. Standard solutions of calcium oxide with varying concentrations (10-100 ppm) were prepared to calibrate the system for the measurement of calcium [43]. The concentration of calcium was expressed in mg/g.

5.2.14 Statistical analysis

All experiments were performed in triplicate, and the obtained results are expressed as the mean values and standard deviations. Statistical difference was evaluated by using ANNOVA test in SPSS v.21 and PCA in OriginPro software.

5.3 RESULTS

5.3.1 PHYSICAL PROPERTIES OF FRUITS

In Table 5.2 provides the physical characteristics of fifteen wild edible fruits found in Manipur, including weight, circumference, length, and shape. Most of the fruits had spherical to oval shapes, except for *Averrhoa carambola* and *Garcinia xanthochymus*, which had a distinct star and pear shape, respectively. 100 fruits were taken into account to estimate the weight of the smaller fruit samples, namely *Antidesmus bunius, Phyllanthus emblica, Rhus semialata,* and *Ziziphus mauritiana*, while 50 fruits were used to estimate the weight of the remaining fruit samples. The investigation revealed that *Antidesmus bunius* had the lowest weight at 14.35 ± 3.69 g, while *Garcinia pedunculata* was the heaviest fruit at 629.22 ± 91.23 g, followed by *Dellinia indica* at 540.64 ± 66.49 g. The minimum and maximum circumferences were observed in *Rhus semialata* (0.44 ± 1.22 cm) and *Garcinia pedunculata* (35.56 ± 4.06 cm), respectively. *Antidesmus bunius* had the shortest length at 0.30 ± 0.07 cm, while *Dellinia indica* had the longest length at 15.60 ± 4.02 cm. One-way ANOVA analysis for weight, circumference, and length of the fruit samples showed significant variation in mean-variance, $F_{(2,42)} = 5.285$; $r = 0.009$ ($p \leq 0.05$).

5.3.2 PHYSICO-CHEMICAL PROPERTIES

Table 5.3 presents the physico-chemical properties of fifteen wild edible fruits from Manipur. *A. carambola* (88.09 ± 2.33%) exhibited highest moisture content and lowest was found in *R. semialata* (6.30 ± 1.45%) showing in the order of *A. carambola* > *G. pedunculata* > *D. indica* > *P. emblica* > *S. betaceum* > *E. floribundus* > *P. guajuva* > *F. cunia* > *G. xanthochymus* > *M. paniculata* > *Z. mauritiana* > *V. spinosa* > *A. bunius* > *S. pinnata* > *R. semialata* . Most of the studied fruits had high moisture content except *R. semialata* (Fig. 5.1). However, the total solid content was high in *R. semialata* (93.77 ± 1.45%) and lowest in *A. carambola* (11.96 ± 2.33%). The solubility ranged from 16.67 %– 53.33%, with the lowest and

highest seen in *S. betaceum* (16.67% ± 11.55%) and *V. spinosa* (53.33% ± 5.77%), respectively. The pH of the fresh and dried samples was acidic, with most fruits showing higher acidity in the fresh state except for *A. carambola* and *Z. mauritiana* (Fig. 5.2). In the case of fresh fruit samples, the pH ranged from 2.41 – 5.82, where *G. pedunculata* (2.41 ± 0.02) showed the most acidic fruit among all the fifteen fruit samples, while *P. guajuva* (5.82 ± 0.02) showed the lowest acidic fruit (Fig. 5.2). On the other hand, the pH of the dried samples ranged from 3.09 to 6.98, where *Z. mauritiana* was the highest acidic fruit and the lowest by *D. indica* (Fig. 5.2). In addition, it is observed that the pH of *A. carambola*, *F. cunia*, and *P. guajuva* was nearly unchanged in both the fresh and dry samples. The percentage of titratable acidity ranged from 0.23 – 4.38%, with the lowest and highest observed in *Z. mauritiana* (0.23 ± 0.01%) and *G. xanthochymus* (4.38 ± 0.02%), respectively (Fig. 5.3).

From the investigation of colour coordinates and indexes, the L^* value of most fruit samples was above 50, indicating colour lightness except for *P. emblica***,** *R. semialata* and *V. spinosa which* were less than 50, indicating darkness, ranged from 39.54 ± 0.94 to 63.43 ± 0.33, with the lowest and highest values contributed by *V. spinosa* and *D. indica,* respectively. All the fruit samples had positive a^* coordinates, indicating redness, ranged from 1.14 ± 0.06 to 90.60 ± 0.20, with the lowest and highest seen in *V. spinosa* and *G. pedunculata,* respectively. Similarly, the b^* value of all the samples was positive contributing yellowness in the fruits' appearance, ranging from 0.35 ± 0.01 to 54.42 ± 1.78 where the lowest was observed in *V. spinosa* and the highest in *G. xanthochymus*. *A. carambola* had the highest *WI* and *G. xanthochymus* had the lowest, ranged from 21.30 ± 0.10 to 50.72 ± 2.03. *G. xanthochymus* (126.82 ± 5.26) had the highest *YI,* while *V. spinosa* (1.27 ± 0.07) had the lowest. On the other hand, *F. cunia* had the highest *BI*, and lowest by *V. spinosa*, ranged from 2.36 ± 0.24 to 48.69 ± 1.24. The graphical representation of colour coordinates (*L*, a*, b**) and derived indices (*WI, YI***,** and *BI*) are shown in Fig. 5.4 and Fig. 5.5, respectively. From the overall observation of colour coordinates and indexes**,** *V. spinosa* contributes the lowest value except in WI.

One-way ANOVA revealed a statistically significant variation in physico-chemical properties at $p \leq 0.05$ ($F_{10,154} = 28.084$; r = 0.000). In addition, Levene's homogeneity of variance test also showed a violation of the assumption indicating statistically significant variance at $p \leq 0.05$ ($F_{10,154} = 2.579$; r = 0.006). Table 5.4 demonstrates the Pearson's correlation between the parameters of physico-chemical properties such as moisture, total solids, solubility, pH, titratable acidity, CIELab coordinates (L^*, a^*, b^*) and derived indexes (*WI*, *YI*, *BI*). Moisture content showed statistically perfect negative correlation with total solid at p = 0.01 with r = -1.00 and positive correlation with L^* at p = 0.01 with r = 0.431. Total solid showed negative correlation with *L** at p = 0.01 with r = 0.431. pH showed significant negative correlation with titratable and *a** with r = -0.608 and r = -0.493 respectively at p = 0.01. Solubility showed no correlation with other Physico-chemical parameters. Titratable acidity showed statistically positive correlation with *a**, *b**, and *YI* with r value 0.414, 0.757, and 0.739 respectively at p = 0.01, and negatively correlate with *WI* at p = 0.01 with r = 0.387. The *L** colour coordinated showed a positive correlation with *b** at p = 0.01 with r = 0.406, while L* also showed a positive correlation with *YI* and *BI* at p = 0.05 with an r-value of 0.325 and 0.310, respectively. Meanwhile, *b** showed negative correlation with *WI* at p = 0.001 (r = 0.497), and significant positive correlation with *YI* (r = 0.992) and *BI* (r = 0.623) at p = 0.01. Lastly, *YI* showed negative correlation with *WI* (r = -0.523) and positive correlation with *BI* (r = 0.657) at p = 0.01. Fig. 5.6 represents the biplot (score plot + loading plot) of principal component analysis (PCA) of physico-chemical properties where the PC1 component and PC2 component contribute 34.92% and 20.92%, respectively. The correlation depicted in the Pearson's correlation can relate to the biplot of PCA, which can be used to visualize the relationship between the physico-chemical properties of the fruit samples. The dendrogram in Fig. 5.7 shows that the hierarchical cluster analysis resulted in five clusters. Cluster I included most samples (nine), cluster II included three samples while, clusters III, IV and V included one sample. Each cluster represents the homogeneity of the fruit samples based on the physico-chemical properties.

Table 5.2 Physical characteristics of fifteen wild edible fruits of Manipur, India

Sample	Fresh fruit weight (g)	Min. & Max. (g)	No. of fruits considered	Circumference (cm)	Length (cm)	Shape
Antidesmus bunius	14.35 ± 3.69	8.17 - 20.90	100	0.75 ± 0.13	0.30 ± 0.07	Globose
Averrhoa carambola	134.69 ± 8.87	120.51 - 155.84	50	15.28 ± 2.64	10.56 ± 2.14	Star shape
Dellinia indica	540.64 ± 66.49	444.76 - 650.05	50	31.72 ± 2.47	15.60 ± 4.02	Round and aggregate
Elaeocarpus floribundus	22.52 ± 2.84	18.1 - 26.99	50	5.89 ± 0.45	4.80 ± 0.43	Oval
Ficus cunia	24.33 ± 6.17	15.53 - 35.87	50	4.68 ± 0.8	4.04 ± 0.71	Rounded to pear shape
Garcinia pedunculata	629.22 ± 91.23	420.37 - 773.47	50	35.56 ± 4.06	11.09 ± 1.18	Spherical and segmented
Garcinia xanthochymus	59.82 ± 4.27	48.98 - 70.36	50	15.06 ± 1.22	5.95 ± 0.97	Pear shape
Microcos paniculata	41.35 ± 7.16	30.01 - 54.56	50	7.74 ± 0.95	5.27 ± 0.46	Spherical
Phyllanthus emblica	54.71 ± 10.79	37.81 - 77.63	100	5.10 ± 0.90	2.76 ± 0.59	Globose
Psidium guajuva	75.82 ± 11.81	56.12 - 97.64	50	9.16 ± 0.72	5.80 ± 0.55	Spherical to pear shape
Rhus semialata	16.87 ± 3.1	10.00 - 24.09	100	0.44 ± 1.22	0.31 ± 0.06	Flattened round
Solanum betaceum	38.03 ± 10.46	19.78 - 56.24	50	15.35 ± 1.22	8.06 ± 1.05	Oval shape
Spondias pinnata	31.31 ± 3.57	23.37 - 38.79	50	3.84 ± 0.41	4.67 ± 0.57	Oval shape
Vangueria spinosa	119.81 ± 21.5	81.33 -150.86	50	12.97 ± 1.24	5.94 ± 0.51	Spherical
Ziziphus mauritiana	34.15 ± 4.85	27.07 - 43.23	100	5.16 ± 0.35	3.89 ± 0.45	Globose

Note: Each value is presented in mean ± standard deviation. For the measurement of circumference and length, 20 fruits have been considered for all the samples. However, for the estimation of weight, the number of fruits considered are mentioned in the table.

Table 5.3 Physio-chemical properties of fifteen wild edible fruits

Samples	MC (%)	TS (%)	S (%)	pH		TA (%)	L*	a*	b*	WI	YI	BI
				Fresh	Dry							
Antidesmus bunius	60.79 ± 2.37f	39.21 ± 2.37b	23.33 ± 5.77ed	3.70 ± 0.02hi	4.98 ± 0.01i	0.87 ± 0.02g	57.45 ± 1.2fg	9.93 ± 0.10f	13.91 ± 0.52e	45.95 ± 3.15b	34.58 ± 0.57f	33.62 ± 2.24de
Averrhoa carambola	88.04 ± 2.33a	11.96 ± 2.33g	20.00 ± 10.00de	3.75 ± 0.03gh	3.50 ± 0.02n	1.37 ± 0.03c	63.43 ± 0.33b	11.43 ± 0.88e	16.51 ± 0.23d	50.72 ± 2.03b	37.18 ± 0.32f	36.57 ± 1.25d
Dellinia indica	83.3 ± 0.88b	16.7 ± 0.88f	36.67 ± 20.82abcde	4.07 ± 0.02e	6.98 ± 0.01a	0.60 ± 0.0i	73.4 ± 0.54a	3.02 ± 0.02j	8.35 ± 0.14g	58.06 ± 0.39a	16.25 ± 0.39h	7.96 ± 0.04h
Elaeocarpus floribundus	81.93 ± 0.38bc	18.07 ± 0.38ef	46.67 ± 11.55abc	3.37 ± 0.02k	5.67 ± 0.01f	1.06 ± 0.04d	59.06 ± 0.43ef	7.46 ± 0.11g	18 ± 0.09c	33.39 ± 1.01ef	43.54 ± 0.53de	36.15 ± 2.60de
Ficus cunia	80.97 ± 0.6bc	19.03 ± 0.60ef	30.00 ± 10.00bcde	5.48 ± 0.01b	5.53 ± 0.01g	0.27 ± 0.00lm	53.07 ± 2.14h	12.52 ± 0.84d	19.65 ± 0.59b	38.74 ± 6.71cd	53.00 ± 3.73b	48.69 ± 1.24a
Garcinia pedunculata	86.2 ± 0.36a	13.8 ± 0.36g	26.67 ± 5.77cde	2.41 ± 0.02m	3.91 ± 0.01m	1.94 ± 0.05b	50.97 ± 1.61i	90.6 ± 0.20a	12.47 ± 0.58f	40.39 ± 1.85c	34.94 ± 0.52f	33.28 ± 1.43de
Garcinia xanthochymus	79.43 ± 0.96c	20.57 ± 0.96e	30.00 ± 0.00bcde	2.77 ± 0.06l	4.02 ± 0.01l	4.38 ± 0.02a	61.32 ± 0.54 cd	23.96 ± 1.32b	54.42 ± 1.78a	21.3 ± 0.1g	126.82 ± 5.26a	41.93 ± 1.32c
Microcos paniculata	72.3 ± 1.77d	27.7 ± 1.77d	50.00 ± 20.00ab	3.84 ± 0.02f	6.91 ± 0.03b	0.47 ± 0.03k	56.29 ± 1.52g	13.77 ± 0.48c	17.79 ± 0.88c	47.96 ± 0.5b	45.22 ± 3.46cde	45.22 ± 4.87b
Phyllanthus emblica	82.37 ± 1.88bc	17.63 ± 1.88ef	40.00 ± 10.00abcd	3.42 ± 0.01k	5.90 ± 0.00e	0.82 ± 0.04g	43.71 ± 0.44j	3.9 ± 0.03i	5.67 ± 0.09h	37.5 ± 0.2cde	18.53 ± 0.48h	12.49 ± 0.09g
Psidium guajuva	81.63 ± 1.38bc	18.37 ± 1.38ef	40.00 ± 0.00abcd	5.82 ± 0.02a	5.97 ± 0.00d	0.26 ± 0.00lm	60.05 ± 0.89de	7.12 ± 0.22g	19.45 ± 0.36b	33.32 ± 1.99ef	46.27 ± 0.17cd	33.98 ± 0.86de
Rhus semialata	6.3 ± 1.45g	93.7 ± 1.45a	20.00 ± 10.00de	3.48 ± 0.08j	4.67 ± 0.02k	0.73 ± 0.00h	42.28 ± 0.03j	6.03 ± 0.04h	8.02 ± 0.06g	39.6 ± 1.00c	27.10 ± 0.22g	17.28 ± 0.71f
Solanum betaceum	82.03 ± 1.39bc	17.97 ± 1.39ef	16.67 ± 11.55e	4.50 ± 0.02c	6.82 ± 0.01c	0.30 ± 0.01l	56.44 ± 0.06g	7.47 ± 0.06g	16.66 ± 0.05d	28.35 ± 2.39f	42.17 ± 0.08e	32.93 ± 1.36e

Table 5.3 (Contd.) Physio-chemical properties of fifteen wild edible fruits

Samples	MC (%)	TS (%)	S (%)	pH		TA (%)	L*	a*	b*	WI	YI	BI
				Fresh	Dry							
Spondias pinnata	58.8 ± 3.49f	41.2 ± 3.49b	26.67 ± 5.77cde	4.33 ± 0.02d	4.88 ± 0.02j	0.94 ± 0.01e	62.56 ± 1.11bc	6.57 ± 0.08gh	18.37 ± 0.40c	33.32 ± 2.6ef	41.95 ± 0.17e	33.99 ± 0.17de
Vangueria spinosa	66.07 ± 1.39e	33.93 ± 1.67c	53.33 ± 5.77a	3.68 ± 0.01i	5.05 ± 0.02h	0.53 ± 0.01j	39.54 ± 0.94k	1.14 ± 0.06k	0.35 ± 0.01i	34.23 ± 0.18de	1.27 ± 0.07i	2.36 ± 0.24i
Ziziphus mauritiana	71.09 ± 0.36d	28.91 ± 0.36d	40.00 ± 20.00abcd	3.76 ± 0.02g	3.09 ± 0.02o	0.23 ± 0.01m	53.85 ± 0.32h	11.73 ± 0.52de	18.13 ± 0.29c	39.85 ± 6.38c	48.10 ± 0.48c	44.65 ± 1.19bc

Each value is reported in mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different at $p \leq 0.05$. (Where MC=moisture content; TS=total solids; S=solubility; TA=titratable acidity; *L**=darkness to lightness; *a**=greenness to redness; *b**=yellowness vs blueness; *WI*=white index; *YI*=yellow index; *BI*=brown index).

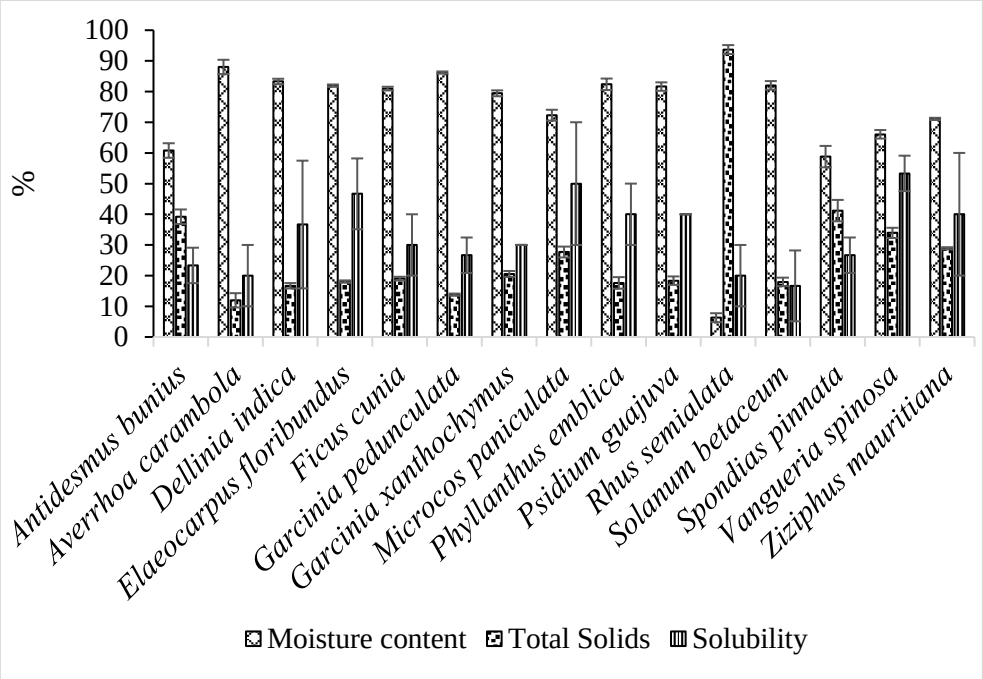

Fig. 5.1 Graphical representation for moisture content, total solids, and solubility percentage of fifteen wild edible fruits

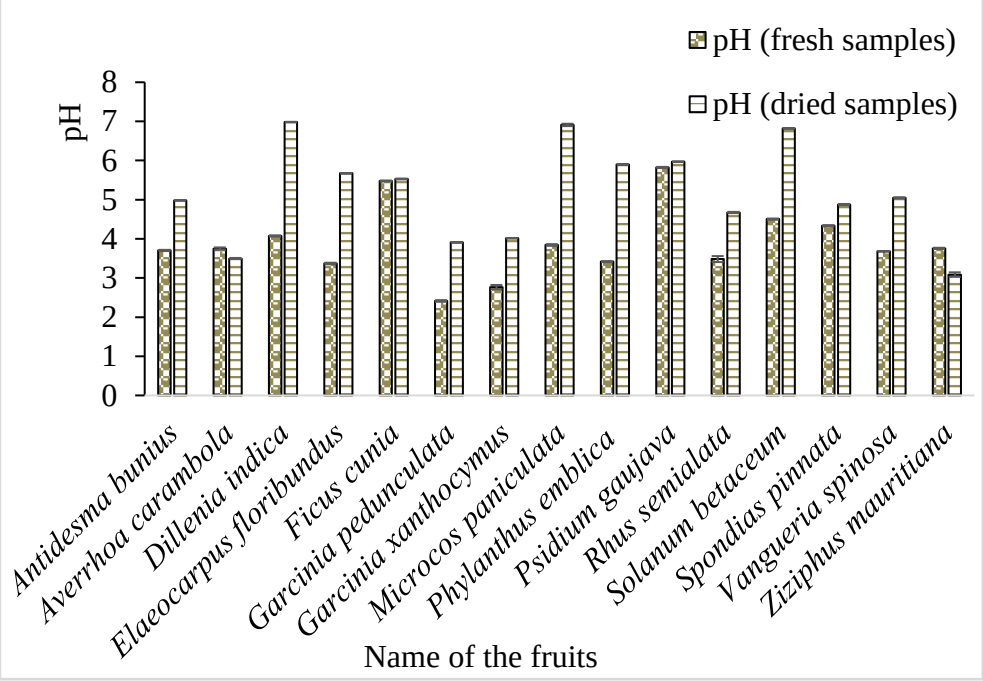

Fig. 5.2 pH of the fifteen fresh and dried wild edible fruits

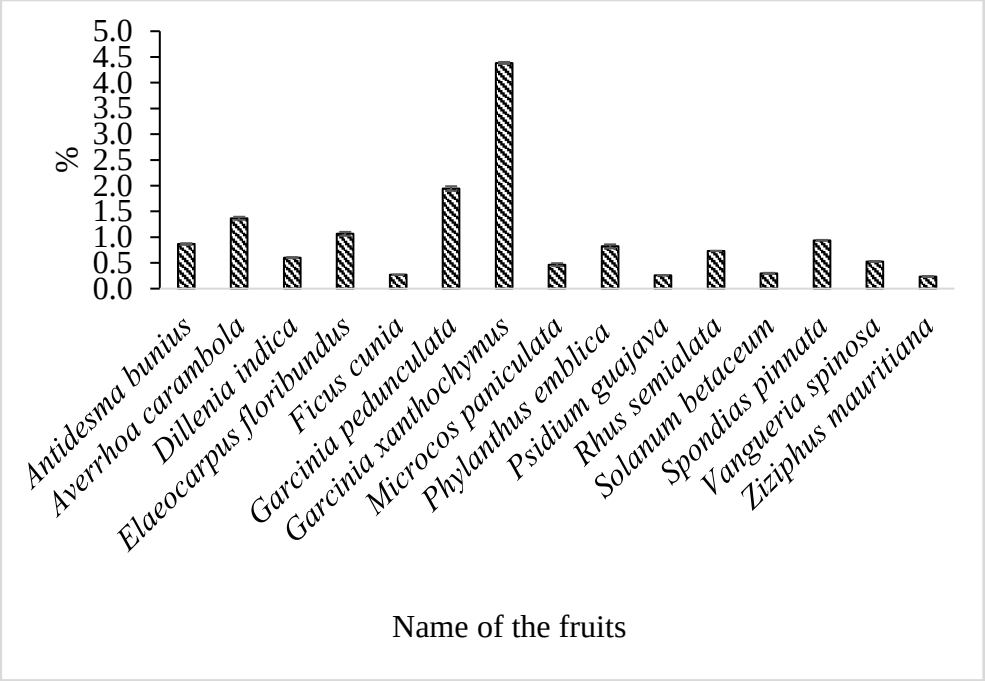

Fig. 5.3 Titratable acidity of the fifteen wild edible fruits

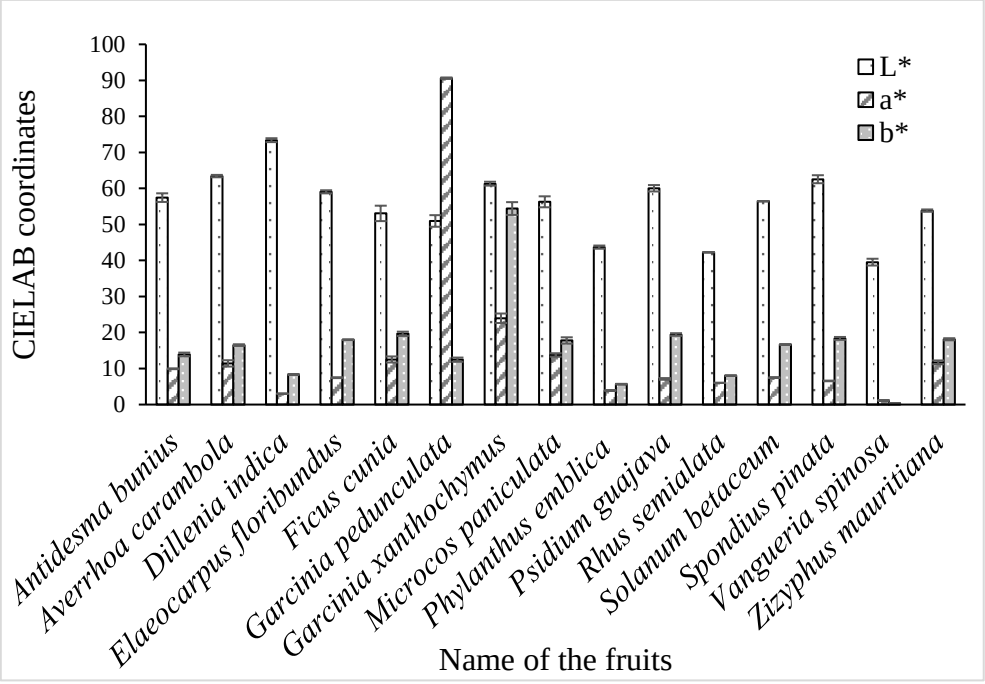

Fig. 5.4 Graphical representation for CIELab colour coordinates (L*=darkness to lightness; a*=greenness to redness; b*=yellowness vs blueness)

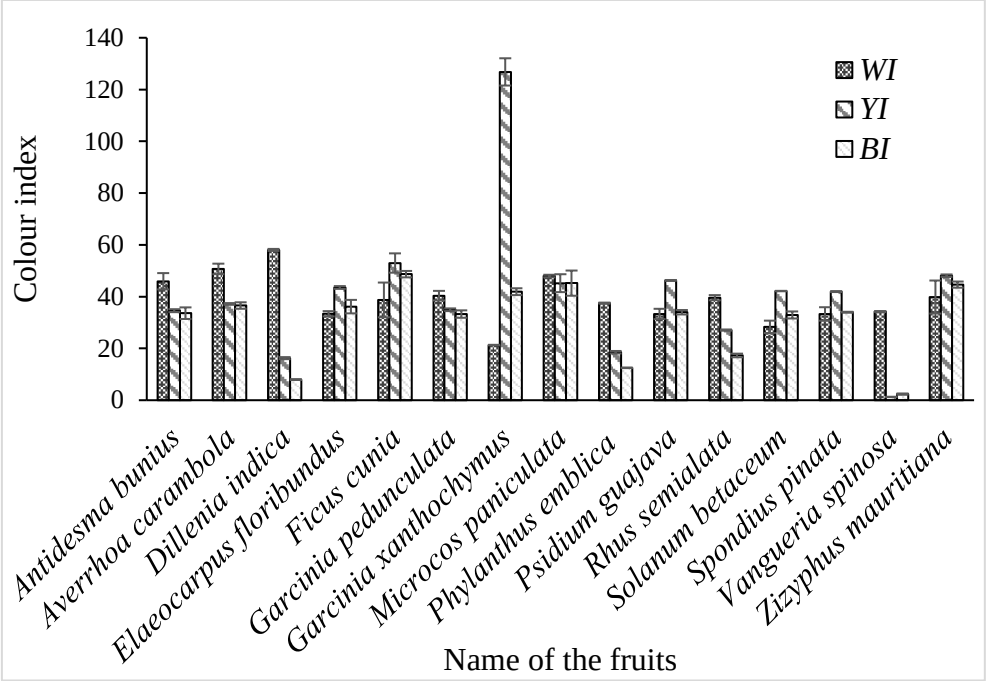

Fig. 5.5 Bar graph showing derived indexes (*WI*= white index; *YI* = yellow index; *BI* = brown index)

Table 5.4 Pearson's correlation of physico-chemical parameters of fifteen wild edible fruits of Manipur

	MC (%)	TS (%)	pH	S (%)	TA (%)	a*	L*	b*	WI	YI
TS (%)	-1.000**									
pH	0.094	-0.094								
S (%)	0.183	-0.183	0.012							
TA (%)	0.138	-0.138	-0.608**	-0.151						
a*	0.230	-0.230	-0.493**	-0.158	0.414**					
L*	0.431**	-0.431**	0.209	-0.164	0.172	-0.070				
b*	0.219	-0.219	-0.102	-0.129	0.757**	0.137	0.406**			
WI	0.016	-0.016	0.015	0.052	-0.387**	-0.028	0.292	-0.497**		
YI	0.175	-0.175	-0.111	-0.135	0.739**	0.172	0.325*	0.992**	-0.523**	
BI	0.248	-0.248	0.171	-0.179	0.173	0.238	0.310*	0.623**	-0.185	0.657**

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

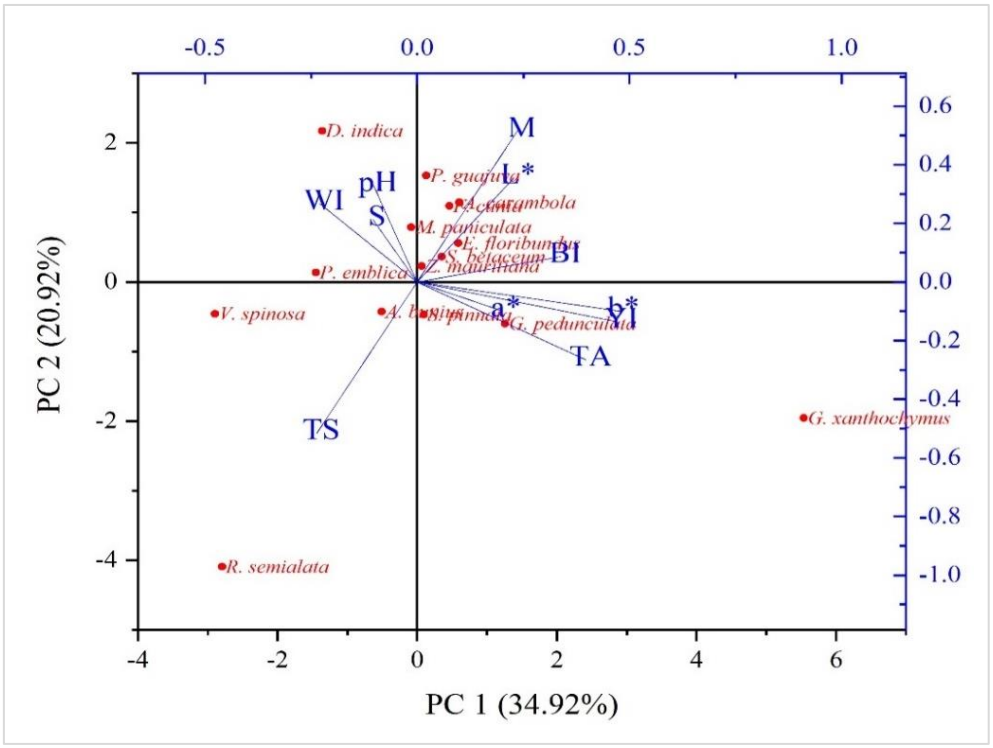

Fig. 5.6 Principal component analysis (PCA) Bi-plot (score and loading plot) of all the parameters of physico-chemical parameters of fifteen fruit samples.

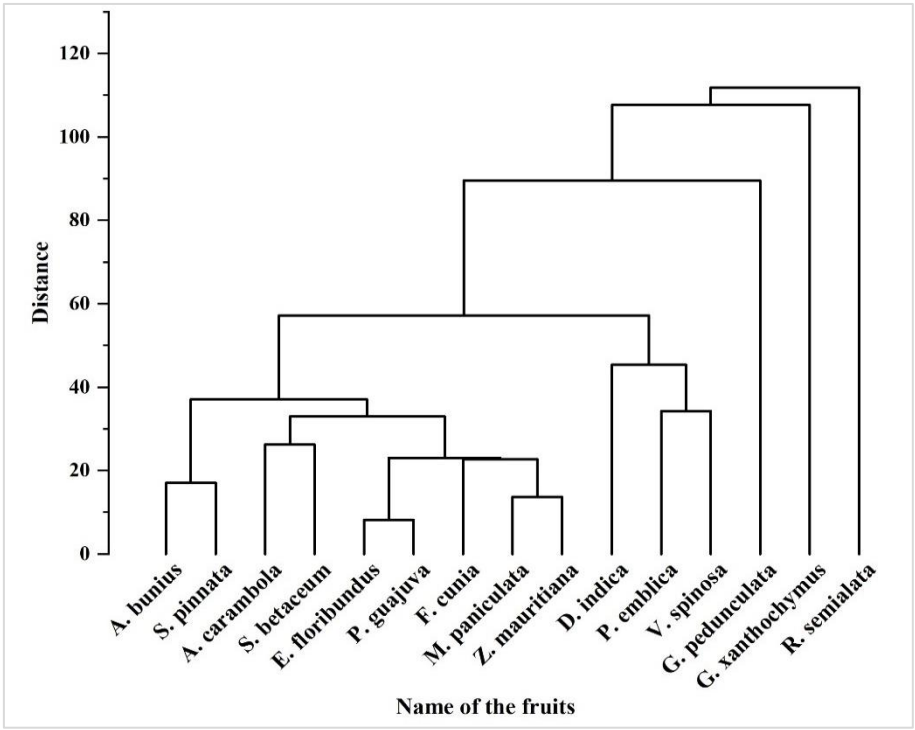

Fig. 5.7 Hierarchical cluster plot for physico-chemical properties of fifteen wild edible fruits

5.3.3 COMPOSITIONAL ANALYSIS

5.3.3.1 Qualitative analysis

In qualitative analysis, aqueous and ethanol extracts were used to screen for various parameters including proteins, carbohydrates, tannins, phenols, flavonoids, saponins, alkaloids, terpenoids, steroids, cardiac glycosides, phlobatannins, anthocyanins, betacyanins, and quinones. The symbols '+++' denoting high concentration, '++' denoting moderate concentration, '+' denoting small concentration, and '-' denoting absence were used to represent the degree of presence or absence of active compounds, as outlined in Tables 5.5 (aqueous extract) and Table 5.6 (ethanol extract).

Based on the analysis, among the four-chemical test of protein estimation for aqueous extract, Million's and Ninhydrin test showed negative in all the fruit samples, while copper sulphate test showed positive for all the studied fruit samples and xanthoproteic test showed positive for the samples *Antidesma bunius, Spondius pinata* and *Vangueria spinosa.* In the case of the solvent (70% ethanol) extract, all the considered chemical test for protein showed positive for at least one sample. Million's test showed positive for in the samples *Dillenia indica, Rhus semialata,* and *Zizyphus mauritian*; Ninhydrin test showed positive only in sample *Spondius pinata*; Copper sulphate test showed positive in most of the samples except *Ficus cunia* and *Solanum betaceum*; Xanthoproteic test showed positive in *Antidesma bunius*, *Averrhoa carambola, Dillenia indica*, *Garcinia xanthochymus, Psidium guajava*, *Spondius pinata,* and *Vangueria spinosa.*

For carbohydrate estimation, almost all the samples showed positive responses in all the three chemical tests, except for *Antidesma bunius* in Benedict's test for the aqueous extract and *Dillenia indica* and *Rhus semialata* in Molisch's test for the solvent extract.

In the tannin test, all samples showed positive results for the solvent extract, while some samples (*Antidesma bunius*, *Garcinia xanthochymus, Psidium guajava*, and *Vangueria spinosa*) showed negative results for the aqueous extract.

Table 5.5 Phytochemical constituents recorded in aqueous extract of fruit sample of the studied species.

Phytoconstituents	Chemical Test	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Proteins	Million's Test	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	Ninhydrin Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Copper Sulphate Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Xanthoproteic Test	-	+	-	-	-	-	+	-	-	+	-	-	+	+++	-
Carbohydrates	Fehling's Test	+	+	+	+	+	+	+	+	+	+	+	+++	+	+	+
	Benedict's Test	-	+	+	+	+++	+	+	+	+	+	+	+++	+	+	+
	Molisch's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Alcoholic Ferric Chloride Test	-	+	+	+	+	+	-	+	+	-	+	+	+	-	+
Phenols	Aqueous Ferric Chloride Test	-	+	+	+	+	+	-	+	+	-	+	+	+	-	+
Flavanoids	Alkaline reagent Test	+	+++	+	+	+	+	+	+	+++	+++	+	+	+++	+	+
	Lead Acetate Test	-	-	+	+	+	+	-	+	+	-	+	+	+	-	+
Saponins	Foam Test	-	+	-	-	-	++	+	-	+	+	-	+++	+	-	+
Alkaloids	Mayer's Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Wager's Test	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Table 5.5 (Contd.) Phytochemical constituents recorded in aqueous extract of fruit sample of the studied species.

Phytoconstituents	Chemical Test	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Terpenoids	Salkawski Test	-	+	+	-	+	-	-	+	-	-	-	-	-	+	+
Steroids	Salkawski Test	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Cardiac glycosides	Keller-Killiani Test	-	+++	+	+	+	-	+++	+	+	+	-	+	+	+	+
Phlobatannins	Hydrochloric Test	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Anthocyanins	Sodium hydroxide Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Betacyanins	Sodium hydroxide Test	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+
Quinones	Sulphuric acid test	-	+++	-	-	-	-	+	-	-	+	-	+	+++	+++	-

Table 5.6 Phytochemical constituents recorded in ethanol extract of fruit sample of the studied species.

Phytoconstituents	Chemical Test	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Proteins	Million's Test	-	-	+++	-	-	-	-	-	-	-	+	-	-	-	+
	Ninhydrin Test	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	Copper Sulphate Test	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
	Xanthoproteic Test	+	+	+	-	-	-	+	-	-	+	-	-	+	+	-
Carbohydrates	Fehling's Test	+	+	+	+	+	+	+	+	+++	+	+	+	+	+	+
	Benedict's Test	+	+	+	+	+	+	+	+	+++	+	+	+	+	+	+
	Molisch's Test	+	+	-	+	+	+	+	+	+	+	-	+	+	+	++
Tannins	Alcoholic Ferric Chloride Test	+	+	+	+	+	+	+	+	+	+	+	+	+++	+	+
Phenols	Aqueous Ferric Chloride Test	+	+	+	+	-	+	+	+	+	+	+	-	+++	+	+
Flavanoids	Alkaline reagent Test	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+
	Lead Acetate Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Foam Test	+	+	+++	+	-	-	+	+++	-	+	-	++	+	+	-
Alkaloids	Mayer's Test	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+
	Wager's Test	+	+	+	-	-	-	+	-	-	+	-	-	+	+	-
Terpenoids	Salkawski Test	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Steroids	Salkawski Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	Keller-Killiani Test	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Phlobatannins	Hydrochloric Test	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+
Anthocyanins	Sodium hydroxide Test	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Betacyanins	Sodium hydroxide Test	+	+	-	-	-	-	-	-	-	+	-	-	+	+	-
Quinones	Sulphuric acid test	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+

The presence of phenol for aqueous extract was exhibited in most of the sample except *Antidesma bunius*, *Garcinia xanthochymus*, *Psidium guajava*, and *Vangueria spinosa*, and for ethanol extracts, other than *Ficus cunia* and *Solanum betaceum* showed positive result.

In the case of the flavonoid test using aqueous extracts, all the samples showed positive results in the alkaline test while some samples (*Antidesma bunius*, *Averrhoa carambola*, *Garcinia xanthochymus*, and *Vangueria spinosa*) showed negative results in the lead acetate test. However, in the ethanol extract, lead acetate test showed positive in all the samples while in alkaline test, *Elaeocarpus floribundus*, *Garcinia pedunculata*, *Garcinia xanthochymus*, and *Rhus semialata* gives negative results.

Seven (*Averrhoa carambola*, *Garcinia pedunculata*, *Garcinia xanthochymus*, *Phylanthus emblica*, *Psidium guajava*, *Solanum betaceum*, and *Spondius pinata*) of the fifteen aqueous extracts showed the presence of saponin. While, in the case of ethanolic extracts, ten of them showed positive reaction such as *Antidesma bunius*, *Averrhoa carambola*, *Dillenia indica*, *Elaeocarpus floribundus*, *Garcinia xanthochymus*, *Microcos paniculata*, *Psidium guajava*, *Solanum betaceum*, *Spondius pinata*, and *Vangueria spinosa*.

When examining the aqueous extract for alkaloids, solely the *Dillenia indica* sample provided confirmation of the existence of alkaloids via Wager's test. In contrast, the ethanol extracts exhibited positive results for the *Dillenia indica*, *Microcos paniculata*, and *Rhus semialata* samples using Mayer's test, while *Antidesma bunius*, *Averrhoa carambola*, *Dillenia indica*, *Garcinia xanthochymus*, *Psidium guajava*, *Spondius pinata*, and *Vangueria spinosa* displayed positive responses in Wager's test.

Averrhoa carambola, *Dillenia indica*, *Ficus cunia*, *Microcos paniculata*, *Vangueria spinosa*, and *Zizyphus mauritiana* responded positive result in terpenoids test using aqueous extracts of the samples while, all the sample except *Rhus semialata* showed positive results for ethanol extracts.

The presence of steroids was seen only in the sample *Rhus semialata* of aqueous extract.

Most of the samples indicated the existence of cardiac glycosides in both the aqueous and ethanol extracts. Nonetheless, *Antidesma bunius*, *Garcinia pedunculata*, and *Rhus semialata* displayed negative outcomes for the aqueous extract, while *Antidesma bunius* and *Garcinia xanthochymus* yielded negative results for the ethanol extract.

In the aqueous extracts, only Dillenia indica and Solanum betaceum confirmed the presence of phlobatannins, while a total of nine samples (Averrhoa carambola, Dillenia indica, Elaeocarpus floribundus, Ficus cunia, Microcos paniculata, Phylanthus emblica, Psidium guajava, Vangueria spinosa and Zizyphus mauritiana) indicated positive results for ethanol extracts.

Anthocyanins were solely detected in the ethanolic extract of *Dillenia indica*. On the other hand, when testing for betacyanin using aqueous extracts, positive outcomes were present in most of the samples except for *Dillenia indica*, *Garcinia xanthochymus*, and *Phylanthus emblica* which yielded negative results. Additionally, for the ethanol extract, *Antidesma bunius*, *Averrhoa carambola*, *Psidium guajava*, *Spondius pinata*, and *Vangueria spinosa* showed positive reactions.

The existence of quinones in the samples' aqueous extracts was evident in *Averrhoa carambola*, *Garcinia xanthochymus*, *Psidium guajava*, *Solanum betaceum*, *Spondius pinata*, and *Vangueria spinosa*. However, ethanol extracts of *Elaeocarpus floribundus*, *Ficus cunia*, *Garcinia pedunculata*, and *Rhus semialata* indicated a lack of quinones.

5.3.4 Quantitative analysis

5.3.4.1 Proximate composition

The proximate composition of the air-dried fruit sample is presented in Table 5.7. The investigation found that ash content ranged from 2.00% to 12.50%. *S. betaceum* had

the highest ash content at 12.50 ± 0.1%, while *G. xanthochymus* had the lowest at 2.00 ± 0.2% of ash content. The fat content was observed between 0.40% and 6.85%, with *E. floribundus* having the lowest fat content and *G. xanthochymus* having the highest. Fig. 5.8 and Fig. 5.9 provide a graphical representation of the ash and fat content in the fruit samples. All fifteen wild edible fruit showed a high calorific value, ranging from 198.48 to 458.59 kcal/100g, with *R. semialata* having the highest and *V. spinosa* having the lowest. (Fig. 5.10). The total carbohydrate and total protein content were estimated by extrapolation of the corresponding calibration curve of total carbohydrate (Fig. 5.11) and total protein (Fig. 5.12). The total carbohydrate content varied from 8.16% to 36.39% (Fig.5.13). The total protein content ranged from 0.70 to 11.66 g100/g (Fig. 5.14). *P. emblica* had the highest protein content, followed by *S. pinnata* (7.39 g/100g), and *E. floribundus* had the lowest value. One-way ANOVA revealed a statistically significant difference between group means ($F_{4,70} = 282.349$; r = 0.000) at p = 0.05. Pearson's correlation (Table 5.9) indicated a statistically significant correlation ($p \leq 0.01$, r = 0.646) between the total carbohydrate content and total protein content.

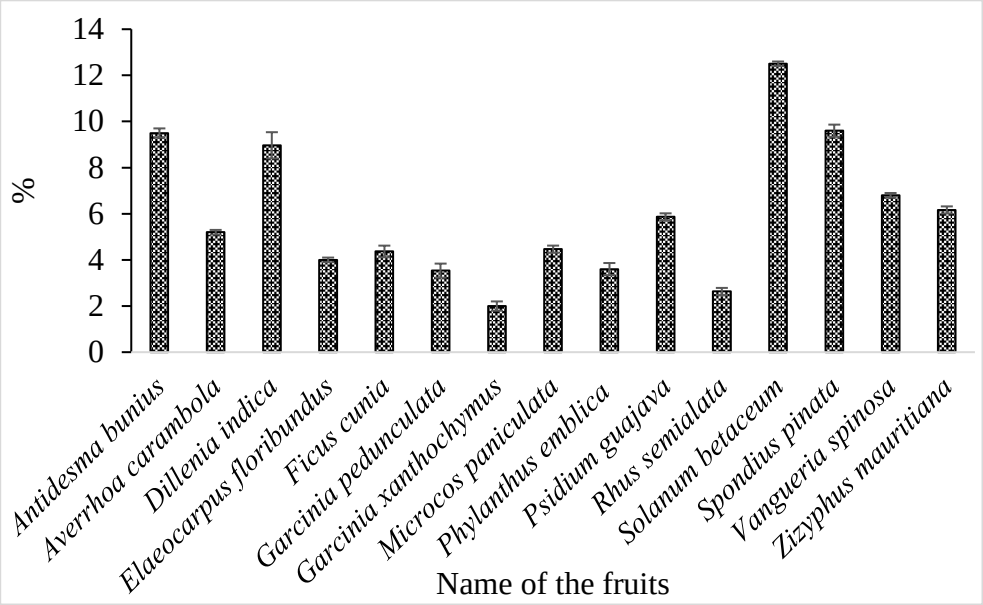

Fig. 5.8 Graphical representation of ash content of fifteen wild edible fruits

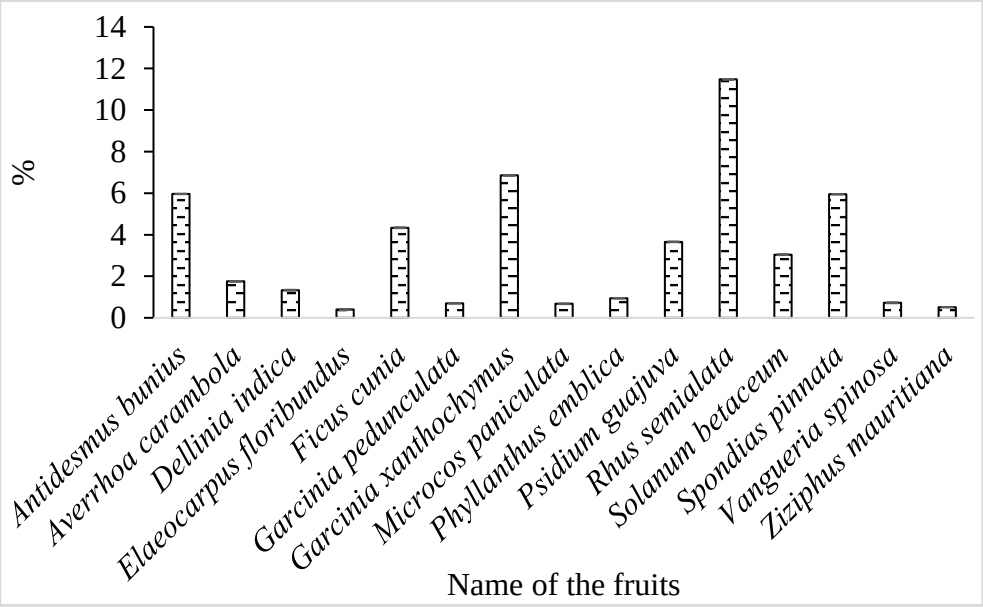

Fig. 5.9 Graphical representation of fat content of fifteen wild edible fruits

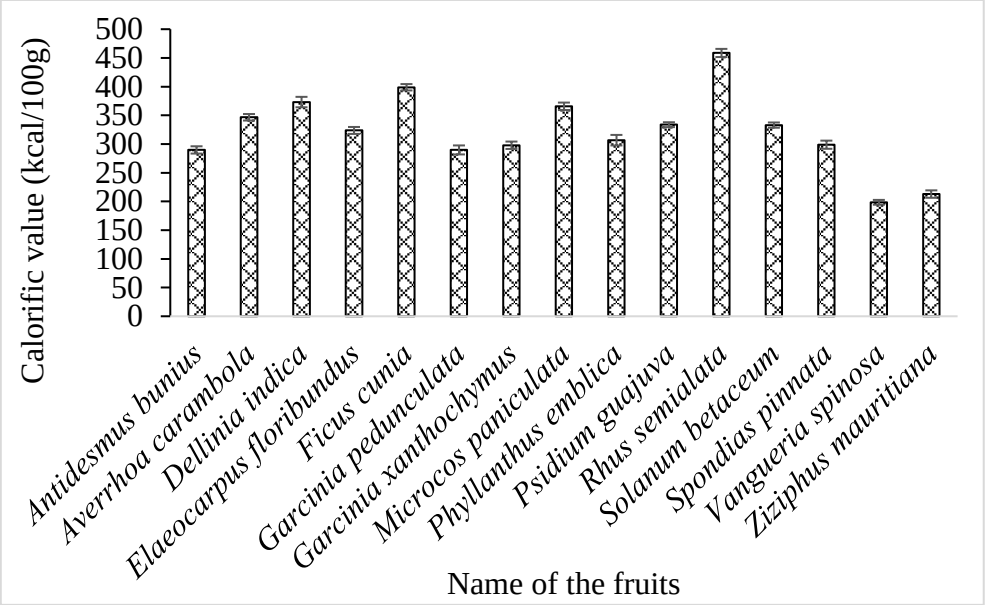

Fig. 5.10 Graphical representation of calorific value of fifteen wild edible fruits

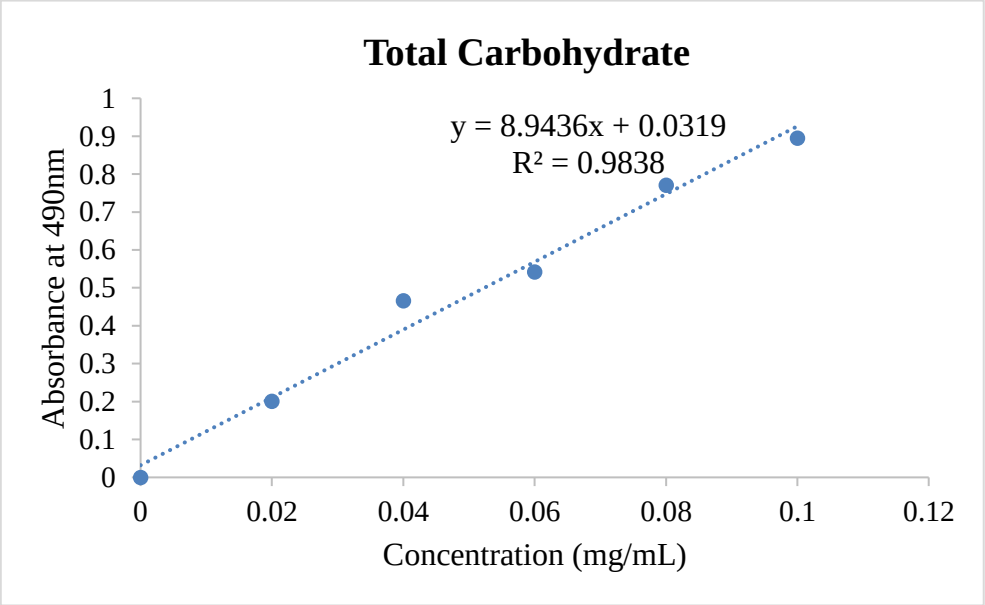

Fig. 5.11 Calibration curve of total carbohydrate content

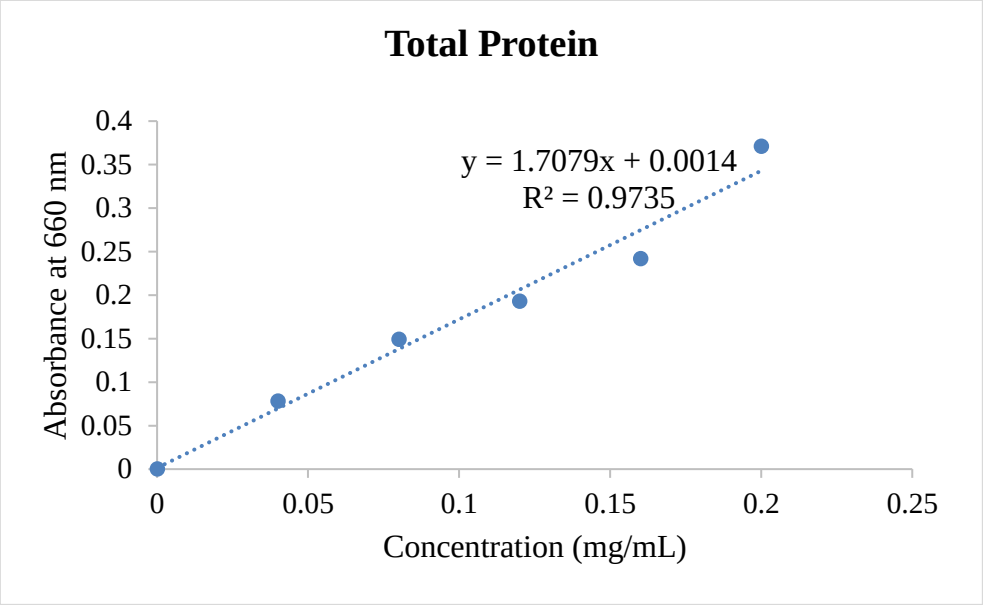

Fig. 5.12 Calibration curve of total protein content

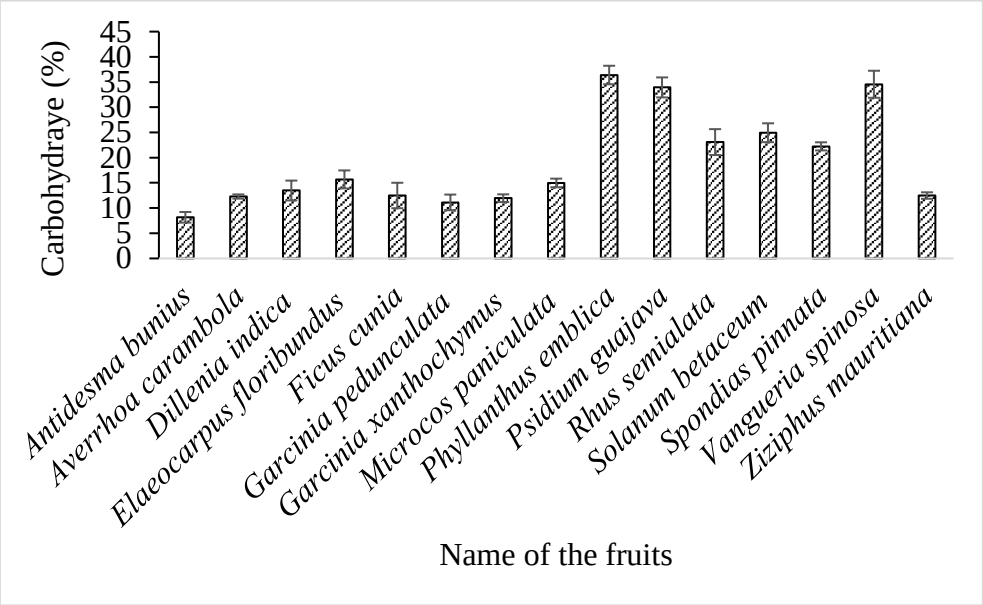

Fig. 5.13 Graphical representation of total carbohydrate content in fifteen wild edible fruits

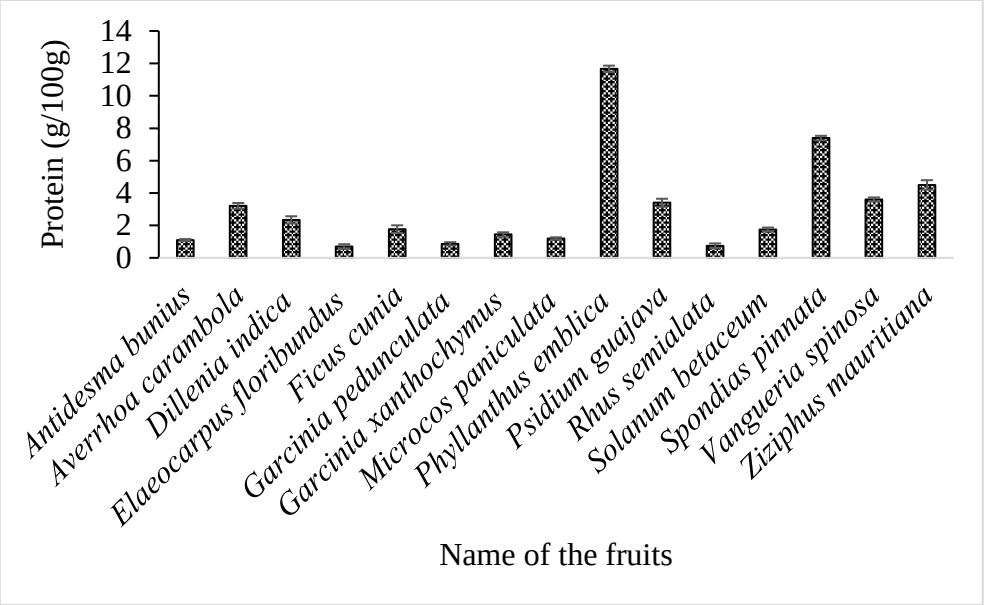

Fig. 5.14 Graphical representation of total protein content in fifteen wild edible fruits

Table 5.7 Proximate composition of fifteen wild edible fruits of Manipur

Sample	Ash content (%)	Fat content (%)	Calorific value (kcal/100g)	Total carbohydrate (%)	Total protein (g/100g)
Antidesmus bunius	9.50 ± 0.2^{b}	5.96 ± 0.01^{c}	289.73 ± 6.36^{g}	8.16 ± 1.06^{e}	1.10 ± 0.06^{ij}
Averrhoa carambola	5.20 ± 0.1^{f}	1.75 ± 0.02^{g}	346.57 ± 5.68^{d}	12.28 ± 0.42^{de}	3.21 ± 0.17^{e}
Dellinia indica	8.97 ± 0.6^{c}	1.33 ± 0.02^{h}	373.08 ± 9.08^{c}	13.48 ± 1.97^{d}	2.34 ± 0.22^{f}
Elaeocarpus floribundus	4.00 ± 0.1^{h}	0.40 ± 0.01^{m}	323.64 ± 6.06^{e}	15.68 ± 1.78^{d}	0.70 ± 0.14^{k}
Ficus cunia	4.37 ± 0.3^{gh}	4.34 ± 0.02^{d}	398.88 ± 5.55^{b}	12.49 ± 2.53^{de}	1.77 ± 0.23^{g}
Garcinia pedunculata	3.53 ± 0.3^{i}	0.70 ± 0.01^{j}	289.73 ± 7.82^{g}	11.11 ± 1.55^{de}	0.86 ± 0.10^{jk}
Garcinia xanthochymus	2.00 ± 0.2^{k}	6.85 ± 0.02^{b}	297.85 ± 6.45^{fg}	11.95 ± 0.77^{de}	1.44 ± 0.13^{h}
Microcos paniculata	4.47 ± 0.2^{g}	0.67 ± 0.02^{k}	365.68 ± 6.47^{c}	14.98 ± 0.86^{d}	1.20 ± 0.07^{hi}
Phyllanthus emblica	3.60 ± 0.3^{i}	0.94 ± 0.01^{i}	306.68 ± 9.20^{f}	36.39 ± 1.83^{a}	11.66 ± 0.20^{a}
Psidium guajuva	5.87 ± 0.2^{e}	3.66 ± 0.02^{e}	333.67 ± 4.26^{e}	33.93 ± 1.98^{b}	3.40 ± 0.25^{de}
Rhus semialata	2.63 ± 0.2^{j}	11.47 ± 0.02^{a}	458.59 ± 7.09^{a}	23.08 ± 2.57^{c}	0.75 ± 0.14^{k}
Solanum betaceum	12.50 ± 0.1^{a}	3.04 ± 0.02^{f}	332.96 ± 4.54^{e}	24.94 ± 1.87^{c}	1.76 ± 0.12^{g}
Spondias pinnata	9.60 ± 0.3^{b}	5.95 ± 0.01^{c}	298.80 ± 7.11^{fg}	22.24 ± 0.80^{c}	7.39 ± 0.17^{b}
Vangueria spinosa	6.80 ± 0.1^{d}	0.72 ± 0.02^{j}	198.48 ± 4.18^{i}	34.56 ± 2.68^{ab}	3.61 ± 0.12^{d}
Ziziphus mauritiana	6.17 ± 0.2^{e}	0.52 ± 0.01^{l}	212.82 ± 6.48^{h}	12.48 ± 0.64^{de}	4.50 ± 0.29^{c}

Each value is reported in mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different at $p \leq 0.05$

5.3.4.2 Phytochemical contents

Table 5.8 presents the phytochemical composition, including total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC), of fifteen wild edible fruits, and Fig. 5.15, Fig. 5.16, and Fig. 5.17 depicted calibration curves. The investigation revealed that the TPC was relatively lower than the flavonoid and tannin contents in most fruit samples (Fig. 5.18), ranging from 0.90 to 5.51 mgGAE/g. *M. paniculata* had the highest phenol content (5.51 ± 0.22 mgGAE/g), followed by *R. semialata* (4.92 ± 0.22 mgGAE/g), while *V. spinosa* had the lowest (0.90 ± 0.04 mgGAE/g). The TFC ranged from of 4.34 to 183.90 mgQE/g, with *P. emblica* having the highest (183.90 ± 4.47 mgQE/g), followed by *D. indica* (94.09 ± 1.13 mgQE/g) and *S. pinnata* (87.74 ± 1.82 mgQE/g), while *G. pedunculata* had the lowest (4.34 V 0.16 mgQE/g). Comparatively, the presence of flavonoids in *P. emblica* showed relatively higher than in the other fruit sample. The TTC ranged from 15.71 – 76.74 mg TAE/g, with *A. carambola* having the highest value (76.74 ± 0.53 mg TAE/g), followed by *S. pinnata* (67.63 ± 0.97 mg TAE/g), and *A. bunius* having the lowest (15.71 ± 0.34 mg TAE/g). One-way ANOVA analysis revealed a significant difference between group means ($F_{2,24} = 6.981$; $r = 0.002$) at $p = 0.05$. Table 5.9 displays the results of Pearson's correlation analysis examining the relationship between proximate composition and phytochemical parameters of fifteen wild edible fruits. The analysis indicated a significant correlation between flavonoid and tannin at $p = 0.05$, with an r-value of 0.529. In addition, only flavonoid content showed a correlation with total carbohydrate and total protein at $p = 0.5$, with r-values of 0.565 and 0.845, respectively. Fig. 5.19 illustrates the correlation between proximate composition and phytochemical parameters of the fifteen wild edible fruits, using PCA cluster analysis, where PC1 and PC2 contribute 38.11% and 23.20%, respectively. On the other hand, Fig. 5.20 displays the dendrogram of hierarchical cluster analysis based on proximate composition and phytochemical parameters, which formed two main cluster classes. Cluster I contained most of the samples (14), while only one sample, *P. emblica*, was included in Cluster II.

Table 5.8 Phytochemical properties of fifteen wild edible fruits of Manipur

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg TAE/g)
Antidesmus bunius	1.41 ± 0.15^{i}	3.22 ± 0.33^{j}	15.71 ± 0.34^{i}
Averrhoa carambola	2.99 ± 0.05^{d}	32.15 ± 1.34^{d}	76.74 ± 0.53^{a}
Dellinia indica	5.51 ± 0.50^{a}	94.09 ± 1.13^{b}	44.12 ± 2.15^{d}
Elaeocarpus floribundus	2.10 ± 0.03^{h}	6.93 ± 0.05^{h}	36.37 ± 0.10^{f}
Ficus cunia	4.01 ± 0.05^{cd}	5.74 ± 0.51^{hi}	34.36 ± 0.39^{g}
Garcinia pedunculata	2.17 ± 0.03^{gh}	4.34 ± 0.16^{ij}	27.60 ± 0.44^{h}
Garcinia xanthochymus	2.36 ± 0.02^{gh}	18.28 ± 0.78^{f}	45.26 ± 1.15^{d}
Microcos paniculata	4.23 ± 0.11^{c}	11.50 ± 0.07^{g}	33.85 ± 1.04^{g}
Phyllanthus emblica	5.25 ± 0.09^{a}	183.90 ± 4.47^{a}	54.70 ± 1.93^{c}
Psidium guajuva	2.49 ± 0.22^{fg}	27.88 ± 0.30^{e}	44.48 ± 0.63^{d}
Rhus semialata	4.92 ± 0.21^{b}	26.53 ± 1.27^{e}	38.51 ± 1.25^{e}
Solanum betaceum	3.73 ± 0.28^{d}	16.16 ± 0.20^{f}	39.82 ± 0.87^{e}
Spondias pinnata	2.75 ± 0.25^{ef}	87.74 ± 1.82^{c}	67.63 ± 0.97^{b}
Vangueria spinosa	0.9 ± 0.04^{j}	11.25 ± 0.20^{g}	33.72 ± 0.13^{g}
Ziziphus mauritiana	4.13 ± 0.05^{c}	6.39 ± 0.95^{hi}	27.41 ± 2.10^{h}

Each value is reported in mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different at $p \leq 0.05$. TPC: Total phenolic content; TFC: Total flavonoid content; TTC: Total tannin content

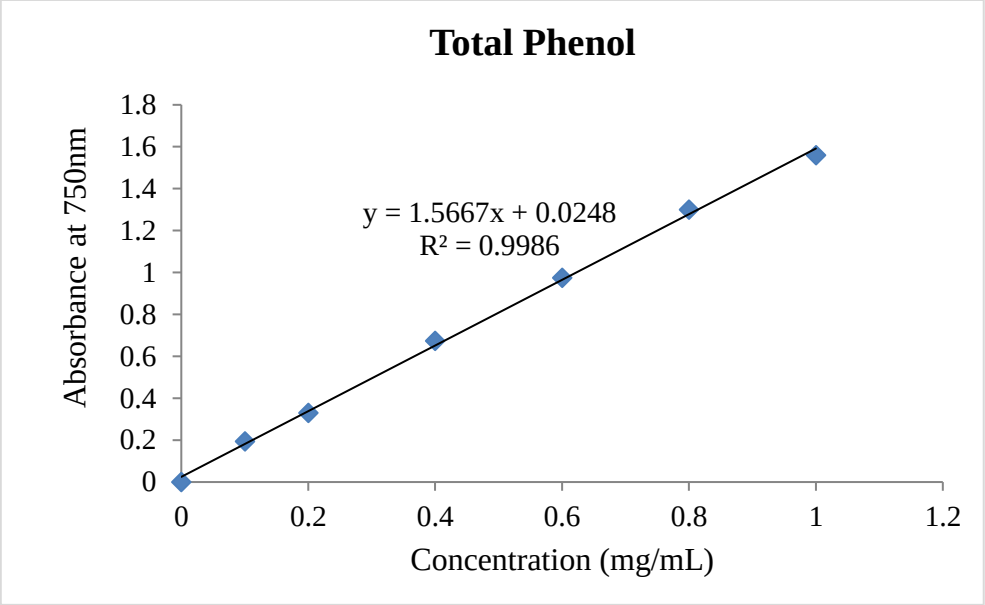

Fig. 5.15 Calibration curve of total phenolics content (TPC)

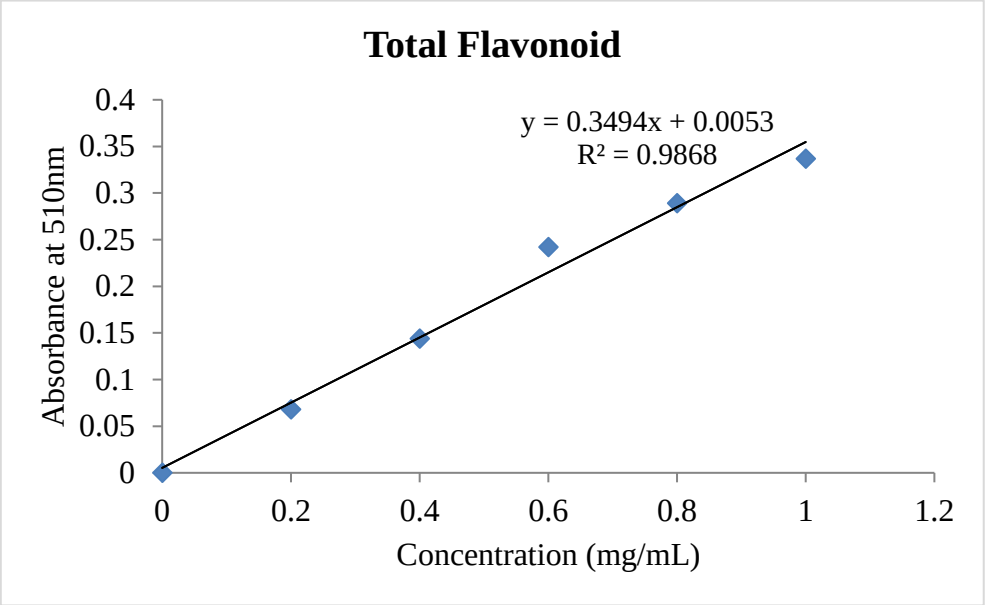

Fig. 5.16 Calibration curve of total flavonoids content (TFC)

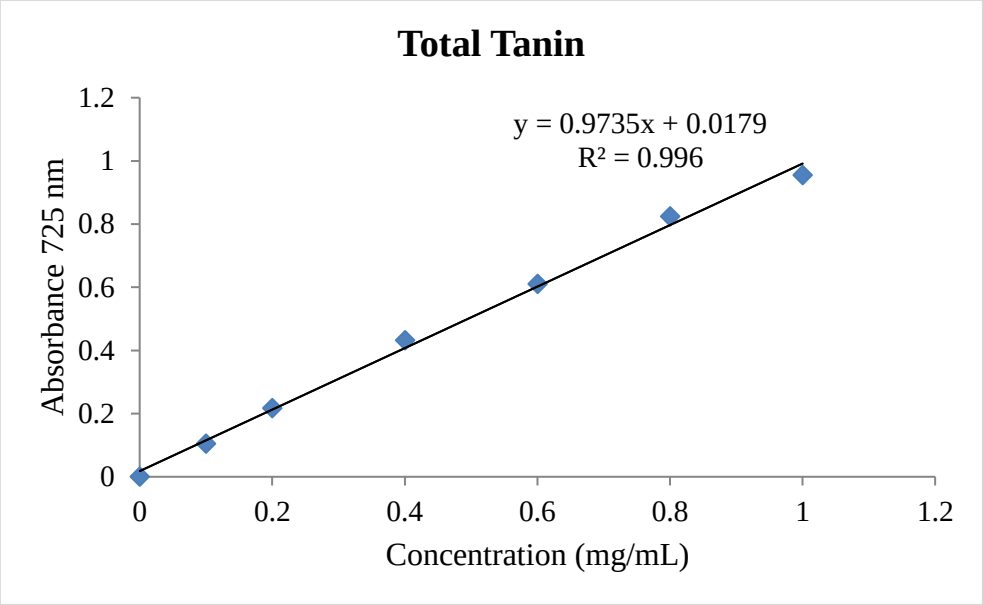

Fig. 5.17 Calibration curve of total tannin content

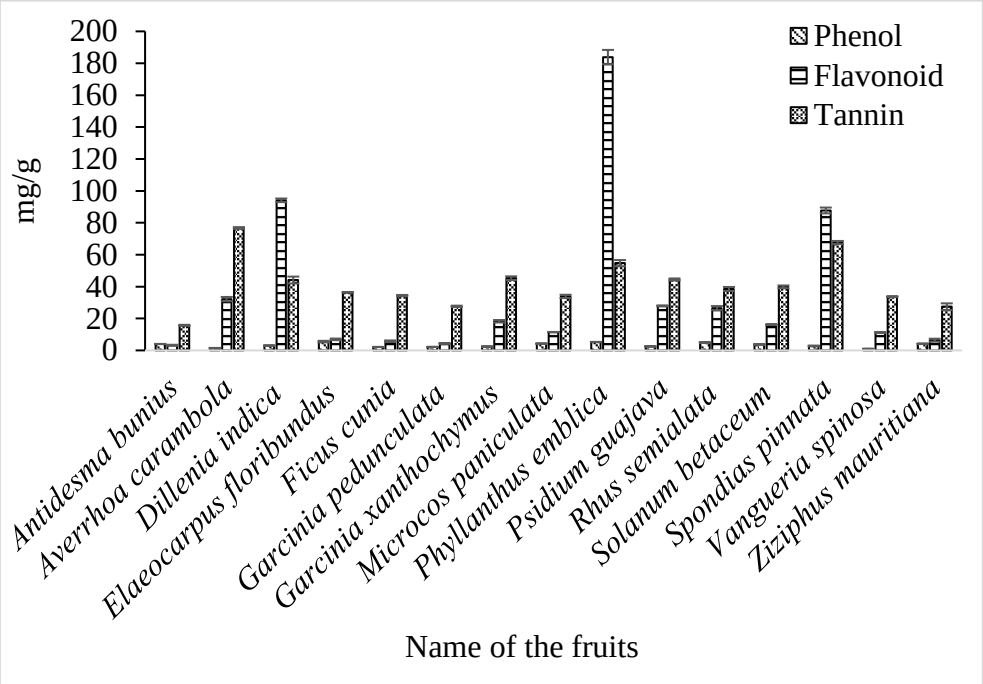

Fig. 5.18 Graphical representation of phytochemical properties

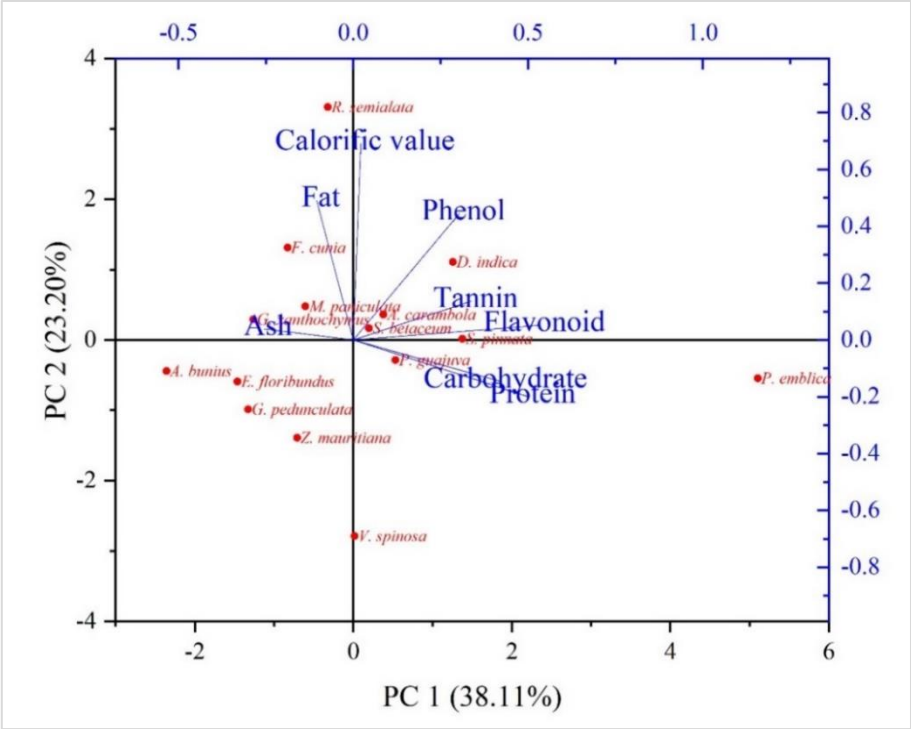

Fig. 5.19 Principal component analysis (PCA) Bi-plot (score and loading plot) of all the parameters of proximate composition and phytochemicals of fifteen fruit samples.

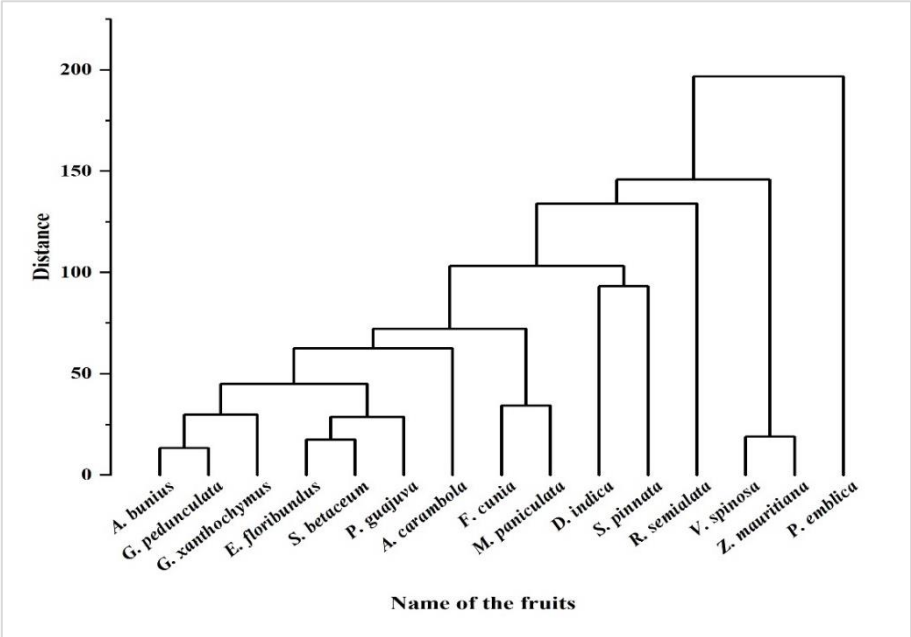

Fig. 5.20 Hierarchical cluster plot based on proximate composition and phytochemical properties of fifteen wild edible fruits.

Table 5.9 Pearson's correlation between proximate composition and phytochemicals

	Ash content	Fat content	Calorific value	Total carbohydrate	Total protein	Phenolic content	Flavonoid content
Fat content	-.087						
Calorific value	-.201	0.492					
Total carbohydrate	.067	-0.048	-0.109				
Total protein	.059	-0.197	-0.301	0.646**			
Phenolic content	-.090	0.087	0.255	0.067	0.117		
Flavonoid content	.031	-0.090	0.069	0.565*	0.845**	0.250	
Tannin content	-.008	0.033	0.181	0.291	0.501	-0.241	0.529*

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

5.3.5 ANTIOXIDANT ACTIVITY

The methanolic extract of fifteen wild edible fruits of Manipur was subjected to three antioxidant assays to evaluate its antioxidant capacity and the results are illustrate in Table 5.10.

5.3.5.1 DPPH Radical Scavenging Activity

The percentage of DPPH radical scavenging effect of the standard ascorbic acid and methanolic extracts is shown in Figs. 5.21 and 5.22, respectively. It is evident from the figure that when the inhibition percentage increases, the concentration of the extracts increases as well. At a concentration of 100 µg/mL, the inhibition percentage of all the wild edible fruit extracts ranged from 14.30% to 60.11% (Fig. 5.22), with the lowest value observed in *A. bunius* and the highest in *P. emblica*. The IC50 value of the standard ascorbic acid was calculated to be 73.72 ± 0.59 µg/mL, while the IC50 values of the fruit methanolic extracts ranged from 78.79 ± 0.14 to 1325.08 ± 11.59 µg/mL, as shown in Fig. 5.23.

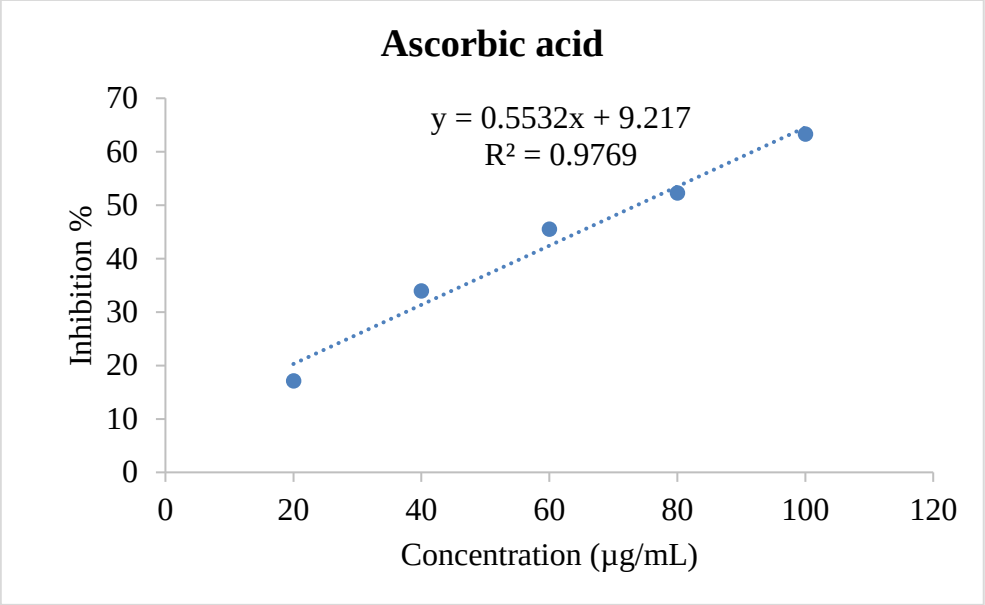

Fig. 5.21 Calibration curve for DPPH radical inhibition % of ascorbic acids

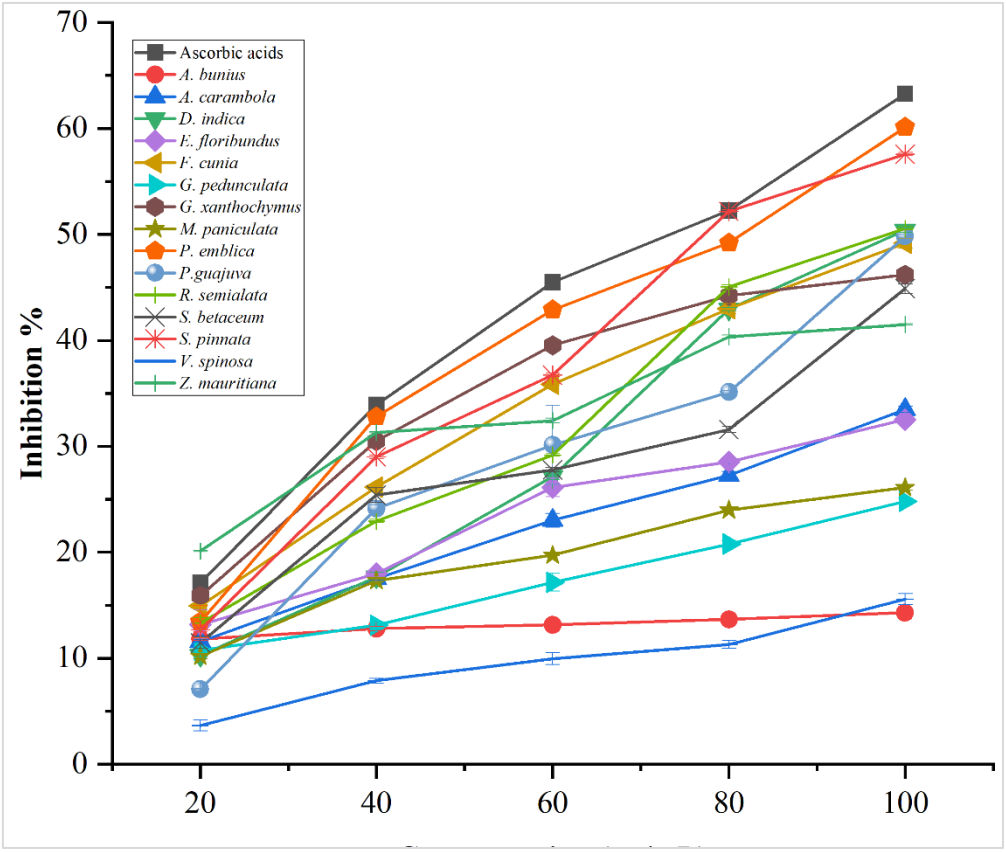

Fig. 5.22 DPPH radical inhibition % of the methanolic extracts of the wild edible fruits (n=3). Error bars denote standard deviation.

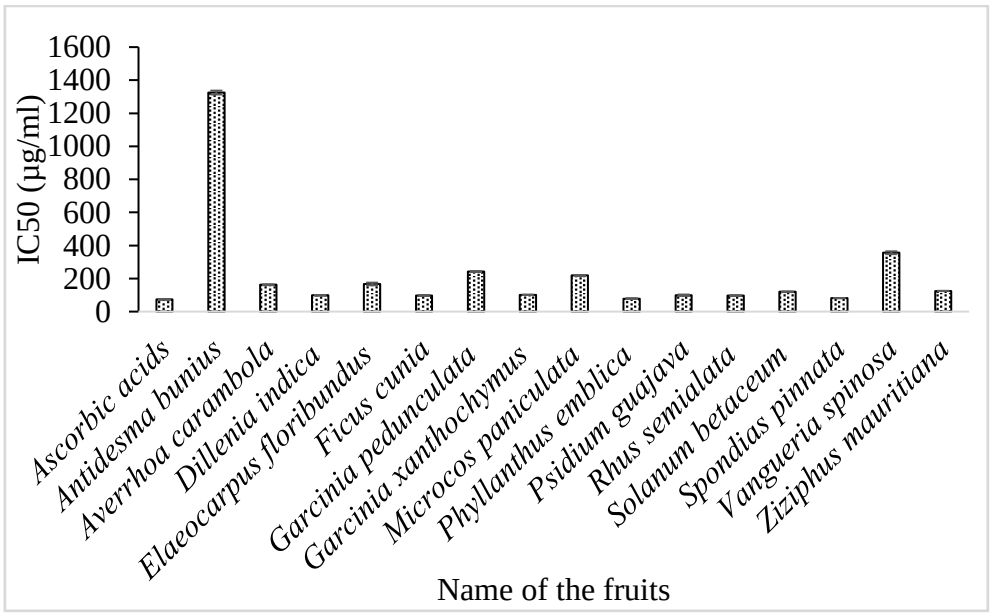

Fig. 5.23 IC_{50} values for DPPH assay (n=3). Error bars denote standard deviation.

Ascorbic acid was used as the standard for the ferric reducing power assay (Fig. 5.24). As illustrated in Fig. 5.25, the FRAP values of wild edible fruits through methanolic extract ranging from 2.23 ± 0.02 to 42.57 ± 0.05 mg/g, with the lowest value observed in *G. pedunculata* and the highest in *P. emblica*.

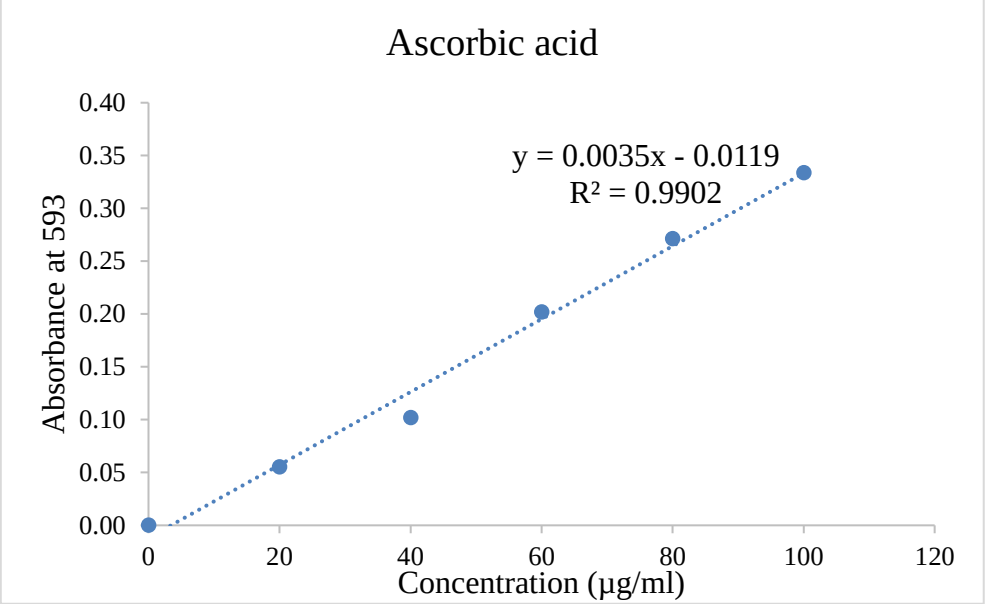

Fig. 5.24 Calibration curve for ferric reducing power of standard ascorbic acid

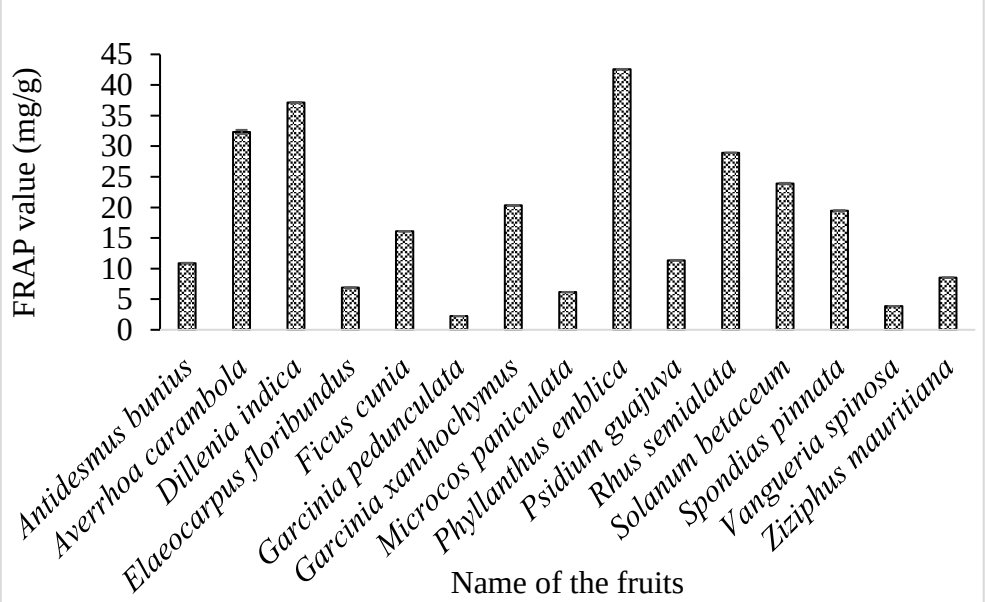

Fig. 5.25 FRAP value for wild edible fruits (n=3). Error bars denote standard deviation.

5.3.5.2 Invitro-antioxidant activity

The in vitro-antioxidant activity of the standard ascorbic acid and methanolic extract of fruit samples are shown in Fig. 5.26 and 5.27, respectively. Fig. 5.28 illustrated the trend of reducing activity of fifteen wild edible fruits. The reducing power of the methanolic extract of fruit samples, as determined by the in vitro antioxidant assay, ranged from 9.09 ± 1.04 to 159.06 ± 4.10 AAE mg/g. The lowest value was observed in *G. pedunculata*, while the highest value was seen in *P. emblica*.

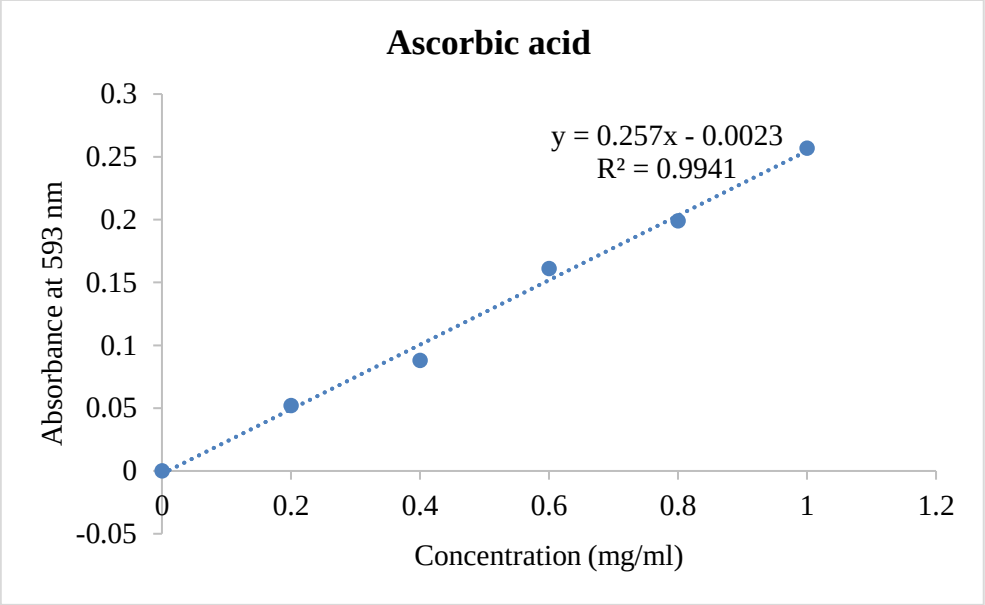

Fig. 5.26 Calibration curve for invitro-antioxidant activity of standard ascorbic acids.

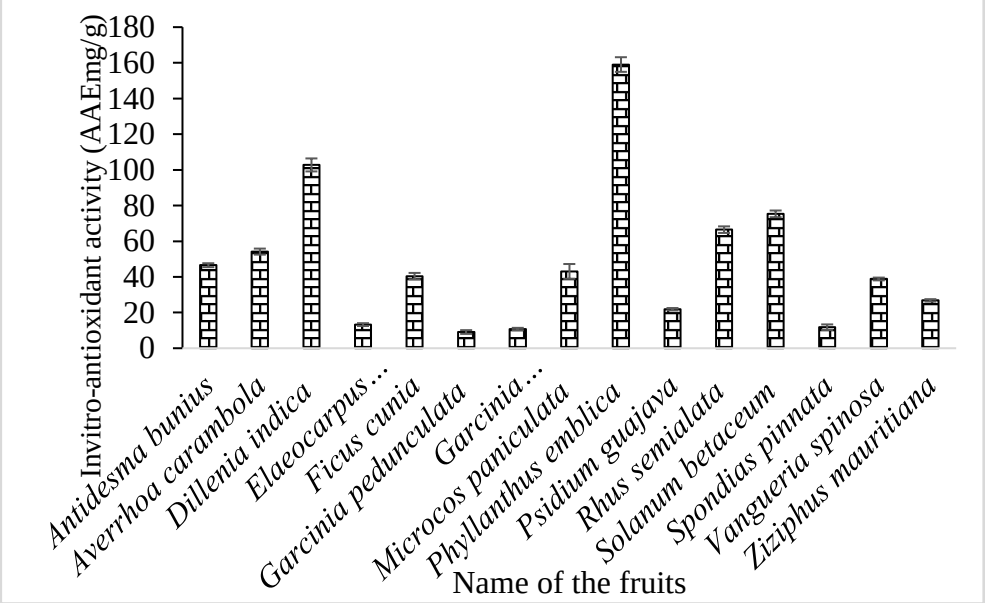

Fig. 5.27 Invitro-antioxidant activity for wild edible fruits (n=3). Error bars denote standard deviation

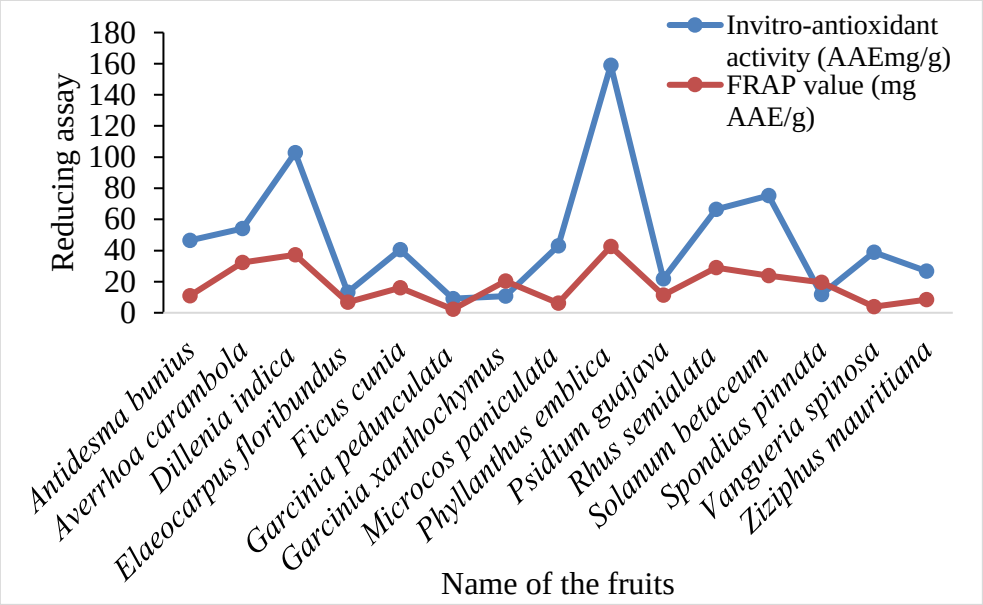

Fig. 5.28 Reducing activity of fifteen wild edible fruits.

Table 5.10 Antioxidant activity of fifteen wild edible fruits of Manipur

Fruits	DPPH IC_{50} (µg/ml)	FRAP value (mg AAE/g)	Invitro-antioxidant activity (mg AAE/g)
Antidesma bunius	1325.08 ± 11.59	10.90 ± 0.04	46.55 ± 1.10
Averrhoa carambola	162.5 ± 2.40	32.32 ± 0.34	54.17 ± 1.69
Dillenia indica	98.55 ± 0.15	37.13 ± 0.03	102.76 ± 9.64
Elaeocarpus floribundus	167.37 ± 7.89	6.89 ± 0.01	13.26 ± 0.79
Ficus cunia	97.96 ± 0.46	16.10 ± 0.04	40.42 ± 1.80
Garcinia pedunculata	242.34 ± 2.34	2.23 ± 0.02	9.09 ± 1.04
Garcinia xanthochymus	101.52 ± 0.94	20.32 ± 0.03	10.77 ± 0.66
Microcos paniculata	218.49 ± 2.95	6.15 ± 0.04	43.08 ± 4.14
Phyllanthus emblica	78.79 ± 0.14	42.57 ± 0.05	159.06 ± 4.10
Psidium guajava	98.9 ± 4.34	11.36 ± 0.06	21.87 ± 0.67
Rhus semialata	96.84 ± 0.25	28.92 ± 0.02	66.48 ± 1.84
Solanum betaceum	119.68 ± 1.72	23.92 ± 0.08	75.34 ± 1.87
Spondias pinnata	81.89 ± 0.12	19.48 ± 0.03	11.82 ± 1.54
Vangueria spinosa	356.08 ± 8.46	3.87 ± 0.02	38.83 ± 0.78
Ziziphus mauritiana	125.13 ± 0.67	8.52 ± 0.04	26.82 ± 0.68

Each value is reported in mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different at $p \leq 0.05$.

Table 5.11 Pearson's correlation coefficient between the antioxidant assays and total phenolic total flavonoid and total tannin contents

	DPPH IC50	FRAP	Invitro-antioxidant activity	TFC	TPC	TTC
DPPH IC50	1					
FRAP	-.299	1				
Invitro-antioxidant acitivity	-.519*	.607*	1			
TFC	-.278	.748**	.529*	1		
TPC	-.492	.671**	.196	.524*	1	
TTC	-.519*	.607*	1.000**	.529*	.196	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

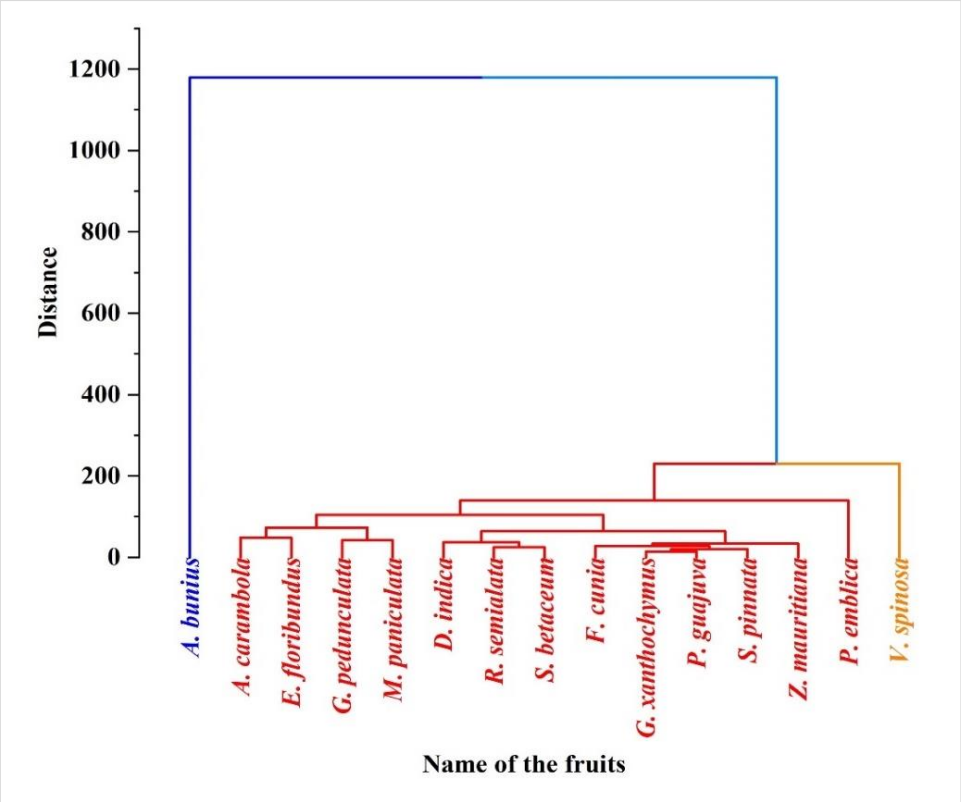

Fig. 5.29 Hierarchical cluster analysis (HCA) plot of fifteen wild edible fruit samples based on the antioxidant activity

The antioxidant activity assays are shown in Table 5.11 with the total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC), revealing significant positive correlations at p = 0.05 and p = 0.01.

DPPH showed significant negative correlation with in vitro-antioxidant activity and TTC at p = 0.05 with same r value (-0.519). However, FRAP showed strong positive correlation with in vitro-antioxidant activity (0.607), TFC (0.748), and TPC (0.671) at p = 0.01, while TTC (0.607) at p = 0.05. In vitro-antioxidant activity displayed perfect positive correlation with TTC at p = 0.01. Furthermore, TFC showed a positive correlation between TPC and TTC at significance level of 0.05.

The hierarchical cluster analysis based on the antioxidant activity of the fifteen wild fruit samples generated a dendrogram with two primary clusters (Fig. 29). Cluster II comprised most of the samples (fourteen) with one outlier (*V. spinosa*), while cluster I included only one sample, *A. bunius*.

5.3.6 VITAMINS

The vitamin C (ascorbic acid), vitamin B1 (thiamine), and vitamin B2 (riboflavin) contents are shown in Table 5.12. Vitamin C was estimated using both fresh and dried samples, and the results are depicted in Fig. 5.30. The vitamin C content of fresh samples ranged from 8.21 to 340.85 mg/100g, while that of dried samples ranged from 0.88 to 8.51 mg/100g. Comparatively, the fresh sample showed higher vitamin C content than the dried sample. *P. emblica* had the highest vitamin C content in both fresh and dried sample, while *A. bunius* and *Z. mauritiana* had the lowest vitamin C content in fresh and dried samples, respectively. It is noted that the fresh fruit samples of *F. cunia* and *G. pedunculata* contained an equal amount of vitamin C (3.81 mg/100g), as did the fresh samples of *M. paniculata* and *R. semialata* (58.08 mg/100g).

Vitamin B_1 (thiamine) and vitamin B_2 (riboflavin) were estimated using extrapolation from the corresponding standard curves of thiamine (Fig. 5.31) and riboflavin (Fig.

5.32). The content of vitamin B_1 and vitamin B_2 ranged from 0.03 – 2.40 mg/100g and 0.25 – 22.15 mg/100g, respectively (Fig. 5.33). The lowest vitamin B_1 and B_2 content was observed in *F. cunia* and *R. semialata*, respectively while the highest vitamin B_1 and B_2 content was found in *V. spinosa*, and *E. floribundus*, respectively. One-way ANOVA analysis for vitamins showed that the difference between group means was statistically significant ($F_{3,56} = 8.194$; $p = 0.000$). Pearson's correlation (Table 5.13) revealed that the content of vitamin C in dried samples was statistically correlated with riboflavin (vitamin B_2) at a confidence level of 0.01 ($r = 0.642$).

Table 5.12 Vitamin C and vitamin B complex content in fifteen wild edible fruits

Sample	Vit. C (mg/ 100g fresh wt.)	Vit. C (mg/ 100g dry wt.)	Vit.B1 (mg/100g)	Vit.B2 (mg/100g)
Antidesma bunius	8.21 ± 1.34^{j}	2.05 ± 0.51^{h}	0.11 ± 0.00^{ij}	0.45 ± 0.01^{k}
Averrhoa carambola	33.73 ± 0.51^{g}	2.35 ± 0.51g^{h}	0.37 ± 0.00^{ef}	0.57 ± 0.02^{j}
Dillenia indica	58.08 ± 0.88^{f}	1.76 ± 0.00^{h}	0.31 ± 0.00^{fg}	0.66 ± 0.06^{h}
Elaeocarpus floribundus	15.25 ± 0.51^{i}	4.40 ± 0.00^{c}	0.11 ± 0.00^{ij}	22.15 ± 0.06^{a}
Ficus cunia	3.81 ± 0.51^{k}	2.93 ± 0.25^{fg}	0.03± 0.00^{j}	0.24 ± 0.01^{m}
Garcinia pedunculata	19.36 ± 1.76^{h}	5.57 ± 0.51^{b}	0.51 ± 0.00^{cd}	2.24 ± 0.03^{d}
Garcinia xanthochymus	3.81 ± 0.51^{k}	3.52 ± 0.00d^{ef}	0.59 ± 0.00^{c}	5.66 ± 0.07^{c}
Microcos paniculata	58.08 ± 0.88^{f}	1.76 ± 0.00^{h}	0.42 ± 0.00^{de}	0.33 ± 0.01^{e}
Phylanthus emblica	340.85 ± 6.60^{a}	8.51 ± 0.51^{a}	0.13 ± 0.02^{hij}	17.85 ± 0.04^{b}
Psidium guajava	246.11 ± 3.56^{b}	2.93 ± 0.51f^{g}	0.16 ± 0.00^{hi}	0.60 ± 0.04^{i}
Rhus semialata	134.93 ± 1.83^{d}	1.76 ± 0.00^{h}	0.54 ± 0.00^{c}	0.25 ± 0.01^{m}
Solanum betaceum	15.25 ± 1.02^{i}	4.11 ± 0.51^{cd}	1.46 ± 0.23^{b}	0.87 ± 0.06^{g}
Spondius pinata	207.97 ± 2.69^{c}	3.81 ± 0.25^{cde}	0.24 ± 0.02^{gh}	1.01 ± 0.01^{f}
Vangueria spinosa	109.71 ± 1.02^{e}	3.23 ± 0.25^{ef}	2.40 ± 0.08^{a}	0.55 ± 0.03^{j}
Zizyphus mauritiana	9.97 ± 0.51^{j}	0.88 ± 0.00h^{i}	0.17 ± 0.01^{hi}	1.57 ± 0.01^{e}

Each value is reported in mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different at $p \leq 0.05$

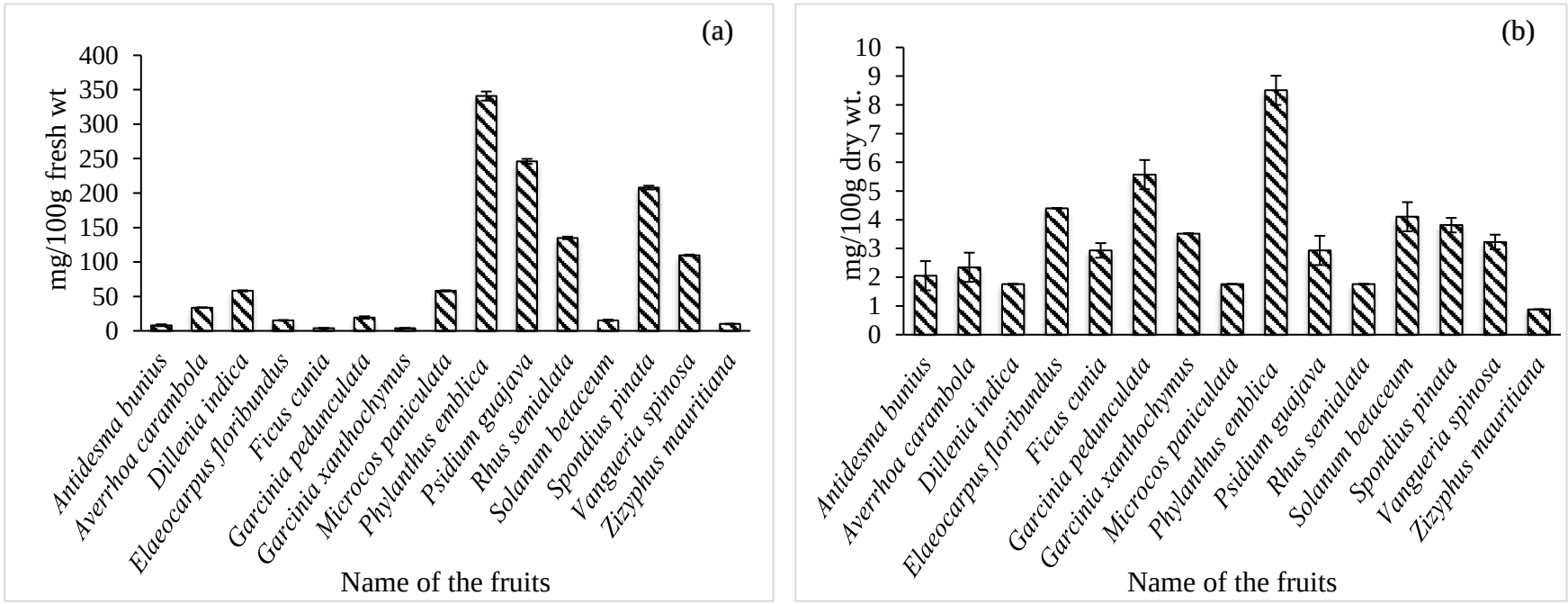

Fig. 5.30 Vitamin C content of the fresh (a) and dried (b) fruit samples (n = 3). Error-bar denote standard deviation

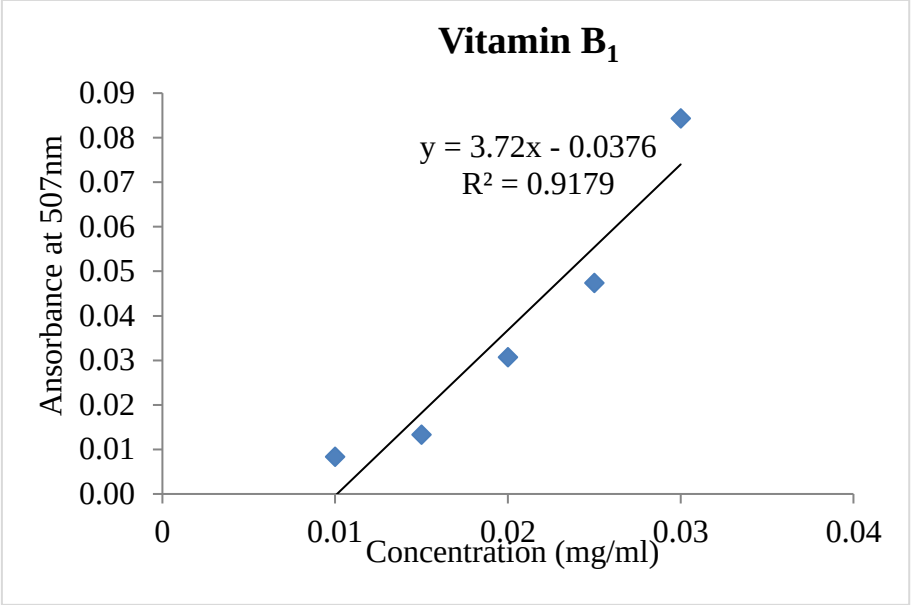

Fig. 5.31 Calibration curve for vitamin B_1 (thiamine)

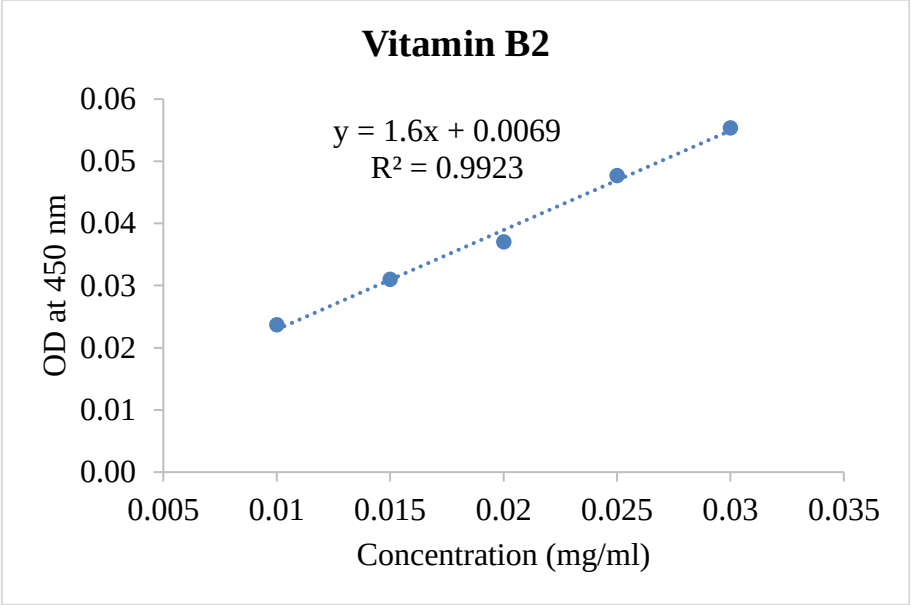

Fig. 5.32 Calibration curve for vitamin B_2 (riboflavin)

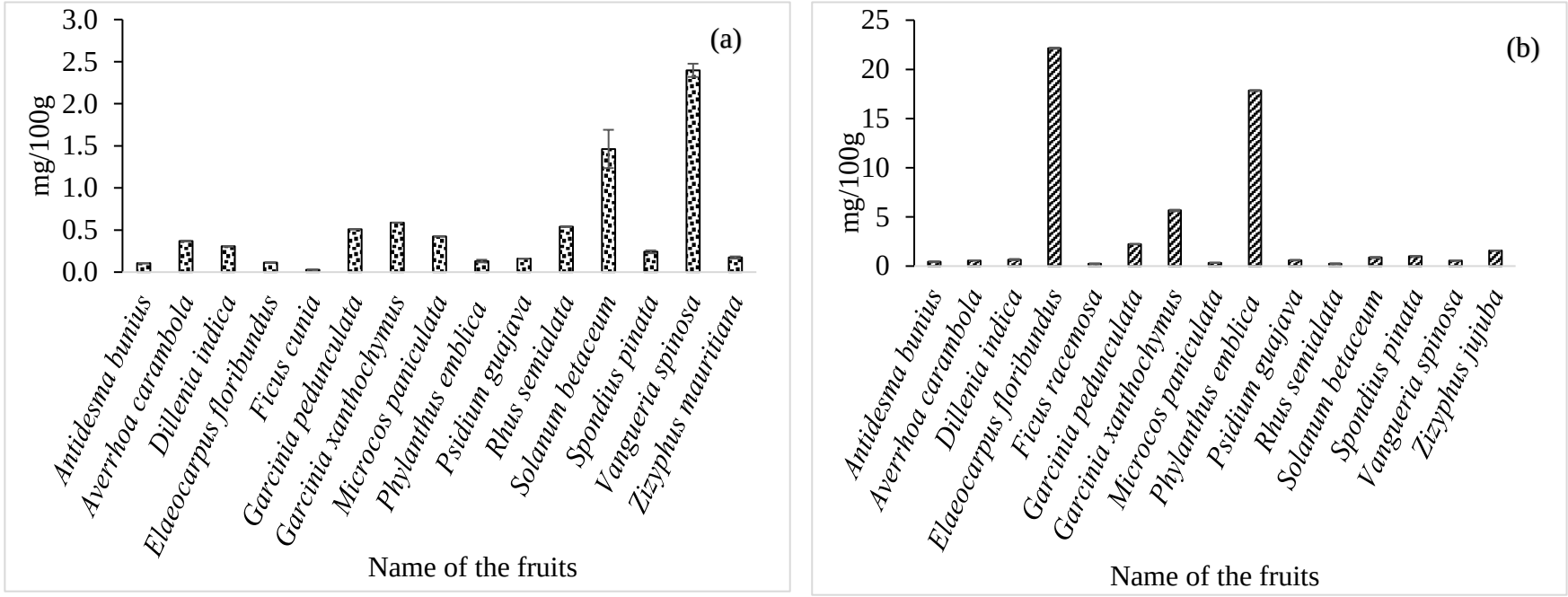

Fig. 5.33 Vitamin B complex content in fifteen wild edible fruit sample (a) vitamin B_1 (b) vitamin B_2 (n = 3). Error-bar denote standard deviation.

Table 5.13 Pearson's correlation coefficient between vitamins and antioxidants

	Ascorbic acid (FW)	Ascorbic acid (DW)	Thiamine	Riboflavin	Invitro-antioxidant	DPPH	FRAP
Ascorbic acid (FW)	1						
Ascorbic acid (DW)	0.499	1					
Thiamine	-.080	.018	1				
Riboflavin	.242	.642**	-.241	1			
Invitro-antioxidant	0.457	0.367	-.009	.228	1		
DPPH	-.257	-.185	-.007	-.154	-.078	1	
FRAP	0.4	.240	-.164	.132	.792**	-0.298	1

**Correlation is significant at the 0.01 level (2-tailed).

5.3.7 MINERAL ELEMENTS

5.3.7.1 Element analysis

A compositional analysis of mineral elements was performed on 15 wild edible fruits using different methodologies, which were classified into two categories: macro-elements and micro-elements. Eight elements were assessed, including four macro-elements (sodium, potassium, magnesium, and calcium) and four micro-elements (iron, copper, manganese, and zinc). Among the studied macro-elements, the potassium content was found to be the highest, ranging from 35.14 – 8738.74 mg/100g. It is observed that the highest and lowest value of potassium content differs drastically, and an equal amount of potassium was seen in four fruit samples viz., *A. carambola* (8738.74 ± 1.88 mg/100g), *D. indica* (8738.74 ± 1.78 mg/100g), *F. cunia* (8738.74 ± 4.33 mg/100g), and *S. betaceum* (8738.74 ± 0.23 mg/100g) as highest content, while the lowest was observed in *G. xanthochymus* (35.14 ± 0.69 mg/100g). The sodium content ranged from 11.67 – 199.27 mg/100g with minimum and maximum values observed in *V. spinosa* (11.67 ± 2.02 mg/100g) and *G. pedunculata* (199.27 ± 0.55) respectively. The magnesium content ranged from 0.09 – 220.74 mg/100g, with the minimum value was contributed by *P. guajuva* (0.09 ± 0.03 mg/100g) and the maximum by *A. bunius* (220.74 ± 1.40 mg/100g). Calcium was

present in the ranged of 16.60 – 93.23 mg/100g, with the lowest and highest amount found in the sample *M. paniculata* (16.60 ± 0.30 mg/100g) and *G. pedunculata* (93.23 ± 6.09 mg/100g), respectively. Additionally, the sodium/potassium ratio was calculated, with *Z. mauritiana* showing the highest value and *G. xanthochymus* showing the lowest.

The iron content was found to be comparatively higher than the other three micro-elements (Cu, Mn, and Zn). The minimum and maximum iron content was observed in *Z. mauritiania* and *F. cunia*, respectively, ranging from 0.11 – 50.67 mg/100g. Copper content ranged from 0.05 – 5.58 mg/100g, with the lowest and highest values observed in samples *M. paniculata* and *D. indica*, respectively. The highest manganese content was found in *P. emblica* (15.79 ± 0.13 mg/100g), while the lowest was in *P. guajuva* (0.12 ± 0.02 mg/100g). The lowest presence of zinc content was observed in *S. pinnata*, while the highest was in the fruit of *A. carambola*, ranging from 0.003 – 39.27 mg/100g. Details of the micro-elements content and macro-elements content of the fifteen wild edible fruits are given in Tables 5.14 and 5.15.

Among the fifteen fruits, seven of them are widely prevalent and commonly consumed by most individuals residing in both the valley and hills of Manipur. These fruits are specifically *Elaeocarpus floribundus, Microcos paniculata, Phylanthus emblica, Psidium guajava, Rhus semialata, Spondius pinnata*, and *Vangueria spinosa*. Regarding the presence of sodium (Na), it was observed that *Phylanthus emblica* exhibited the highest amount at 118.37 ± 1.87 mg/100g, whereas *Vangueria spinosa* displayed the lowest amount at 11.67 ± 2.02 mg/100g. In terms of magnesium (Mg), *Spondius pinnata* demonstrated the highest concentration at 158.62 ± 1.17 mg/100g, while *Psidium guajava* exhibited the lowest concentration at 0.09 ± 0.03 mg/100g. Among these fruits, *Elaeocarpus floribundus* recorded the highest potassium (K) content at 8213.92 ± 2.54 mg/100g, whereas *Psidium guajava* displayed the lowest concentration at 428.56 ± 2.00 mg/100g. *Phylanthus emblica* also exhibited the highest calcium (Ca) content at 83.57 ± 1.01 mg/100g, whereas *Microcos paniculata* displayed the lowest concentration at 16.60 ± 0.30 mg/100g. Moving on to the micro-elements, *Rhus semialata* exhibited the highest amounts of iron (Fe), copper (Cu), and

zinc (Zn) at 19.40 ± 0.04 mg/100g, 1.75 ± 0.005 mg/100g, and 4.13 ± 0.02 mg/100g, respectively. However, *Phylanthus emblica* showcased the highest manganese (Mn) content at 15.79 ± 0.13 mg/100g, while *Psidium guajava* and *Vangueria spinosa* displayed the lowest concentrations at 0.12 ± 0.02 mg/100g and 0.12 ± 0.07 mg/100g, respectively.

One-way ANOVA was performed on all the studied mineral elements, and it was found that the mean difference between group was significant at $p = 0.05$ ($F_{7,112} = 28.021$, $r = 0.000$). Levene's homogeneity test of variance also showed a significant variation between group means at $p = 0.05$ ($F_{7,112} = 69.096$, $r = 0.000$). Meanwhile, Pearson's correlation between the elements is represented in Table 5.16, Sodium showed significant negative correlation with Mg at $p \leq 0.05$, $r = -0.547$, while positively correlate with K ($r = 0.692$), Fe ($r = 0.687$), Cu ($r = 0.726$), and Mn ($r = 0.641$) at 0.01 confidence level. Magnesium was found to be significantly negatively correlate with K at $p \leq 0.01$, $r = 0.665$. At $p \leq 0.05$ potassium was found to be positively correlated with Fe ($r = 0.603$), while at $p \leq 0.01$, K correlated with Fe ($r = 0.603$) and Cu ($r = 0.646$). In the case of calcium element, it is observed that calcium showed statistically no significant relation with other studied elements. Iron was found to be positively correlated with copper at $p \leq 0.05$ with r -value of 0.641. The correlation plot based on principle component analysis (PCA) and hierarchical cluster plot based on macro-elements and micro-elements are presented in Fig. 5.34 and 5.35, respectively.

Table 5.14 Macro-elements content of the fifteen wild edible fruits

Name of the fruits	Na (mg/100g)	Mg (mg/100g)	K (mg/100g)	Ca (mg/100g)	Na/K
Antidesmus bunius	39.40 ± 1.86^{h}	220.74 ± 1.40^{a}	700.36 ± 2.44^{i}	52.27 ± 0.42^{fg}	0.056
Averrhoa carambola	150.95 ± 2.83^{b}	1.03 ± 0.34^{fgh}	8738.74 ± 1.88^{a}	54.00 ± 1.71^{efg}	0.017
Dillenia indica	86.35 ± 2.78^{e}	0.99 ± 0.57^{fgh}	8738.74 ± 1.78^{a}	45.93 ± 3.59^{hi}	0.010
Elaeocarpus floribundus	84.43 ± 1.65^{e}	2.91 ± 0.64^{e}	8213.92 ± 2.54^{b}	45.27 ± 5.28^{hi}	0.010
Ficus cunia	117.08 ± 2.71^{c}	0.78 ± 0.29^{gh}	8738.74 ± 4.33^{a}	77.87 ± 1.23^{c}	0.013
Garcinia pedunculata	199.27 ± 0.55^{a}	0.89 ± 0.59^{fgh}	5339.99 ± 4.23^{f}	93.23 ± 6.09^{a}	0.037
Garcinia xanthochymus	14.30 ± 3.18^{j}	66.16 ± 1.53^{d}	35.14 ± 0.69^{l}	49.27 ± 0.35^{gh}	0.407
Microcos paniculata	90.80 ± 0.67^{d}	1.91 ± 0.74^{efg}	3048.45 ± 3.00^{g}	16.60 ± 0.30^{k}	0.030
Phylanthus emblica	118.37 ± 1.87^{c}	2.27 ± 0.07^{ef}	6803.94 ± 2.12^{c}	83.57 ± 1.01^{b}	0.017
Psidium guajava	24.02 ± 2.09^{i}	0.09 ± 0.03^{h}	428.56 ± 2.00^{k}	43.67 ± 3.03^{i}	0.056
Rhus semialata	74.35 ± 3.23^{f}	2.69 ± 0.06^{e}	5636.28 ± 2.72^{e}	33.80 ± 1.41^{j}	0.013
Solanum betaceum	93.85 ± 2.67^{d}	$0.62 \pm 0.01g^{h}$	8738.74 ± 0.23^{a}	56.03 ± 0.76^{ef}	0.011
Spondius pinnata	23.50 ± 1.45^{i}	158.62 ± 1.17^{b}	666.94 ± 2.80^{j}	61.46 ± 0.30^{d}	0.035
Vangueria spinosa	11.67 ± 2.02^{j}	105.95 ± 1.03^{c}	873.87 ± 1.33^{h}	$58.74 \pm 0.37d^{e}$	0.013
Zizyphus mauritiana	55.06 ± 1.38^{g}	1.62 ± 0.05^{efg}	6139.55 ± 1.02^{d}	37.07 ± 4.91^{j}	0.009

Values reported are mean ± standard deviation (n = 3). Means in the column row with different superscripts are significantly different at $p \leq 0.05$.

Table 5.15 Micro-elements content of the fifteen wild edible fruits

Name of the fruits	Fe (mg/100g)	Cu (mg/100g)	Mn (mg/100g)	Zn (mg/100g)
Antidesmus bunius	1.57 ± 0.11^{i}	0.09 ± 0.01^{j}	5.22 ± 0.06^{d}	0.49 ± 0.10^{j}
Averrhoa carambola	12.44 ± 0.46^{i}	2.44 ± 0.02^{c}	9.76 ± 0.02^{b}	39.27 ± 0.01^{a}
Dillenia indica	20.69 ± 0.15^{b}	5.58 ± 0.09^{a}	2.95 ± 0.01^{j}	2.47 ± 0.01^{f}
Elaeocarpus floribundus	18.07 ± 0.05^{d}	1.73 ± 0.00^{e}	5.33 ± 0.01^{d}	3.23 ± 0.01^{e}
Ficus cunia	50.67 ± 0.12^{a}	2.28 ± 0.01^{d}	5.82 ± 0.14^{c}	3.88 ± 0.03^{c}
Garcinia pedunculata	32.58 ± 0.11^{a}	5.33 ± 0.01^{b}	5.05 ± 0.00^{e}	3.52 ± 0.01^{d}
Garcinia xanthochymus	4.12 ± 0.10^{h}	0.14 ± 0.04^{i}	0.25 ± 0.03^{k}	0.16 ± 0.04^{k}
Microcos paniculata	10.40 ± 0.08^{f}	0.05 ± 0.05^{j}	3.97 ± 0.05^{h}	0.17 ± 0.01^{k}
Phylanthus emblica	15.12 ± 0.07^{e}	1.37 ± 0.04^{g}	15.79 ± 0.13^{a}	1.64 ± 0.03^{h}
Psidium guajava	3.03 ± 0.15^{h}	0.09 ± 0.01^{j}	0.12 ± 0.02^{l}	0.15 ± 0.02^{k}
Rhus semialata	19.40 ± 0.04^{c}	1.75 ± 0.05^{e}	4.23 ± 0.04^{g}	4.13 ± 0.02^{b}
Solanum betaceum	8.79 ± 0.06^{g}	1.62 ± 0.05^{f}	4.68 ± 0.02^{f}	1.76 ± 0.02^{g}
Spondius pinnata	0.96 ± 0.07^{j}	0.13 ± 0.04^{i}	0.17 ± 0.07^{kl}	0.003 ± 0.001^{l}
Vangueria spinosa	0.17 ± 0.10^{k}	0.07 ± 0.01^{j}	0.12 ± 0.07^{l}	0.10 ± 0.07^{k}
Zizyphus mauritiana	0.11 ± 0.02^{k}	0.90 ± 0.05^{h}	3.48 ± 0.14^{i}	1.09 ± 0.01^{i}

Values reported are mean ± standard deviation (n = 3). Means in the same column with different superscripts are significantly different at $p \leq 0.05$

Table 5.16 Pearson's correlation between macro and micro elements

	Na	Mg	K	Ca	Fe	Cu	Mn	Zn
Na	1							
Mg	-.547**	1						
K	.692**	-.665**	1					
Ca	.470	.050	.176	1				
Fe	.687**	-.471	.603**	.467**	1			
Cu	.726**	-.458	.646**	.393	.641**	1		
Mn	.201	-.177	.258	-.063	.028	.005	1	
Zn	.467	-.223	.411	.047	.111	.237	.121	1

**. Correlation is significant at the 0.01 level (2-tailed).

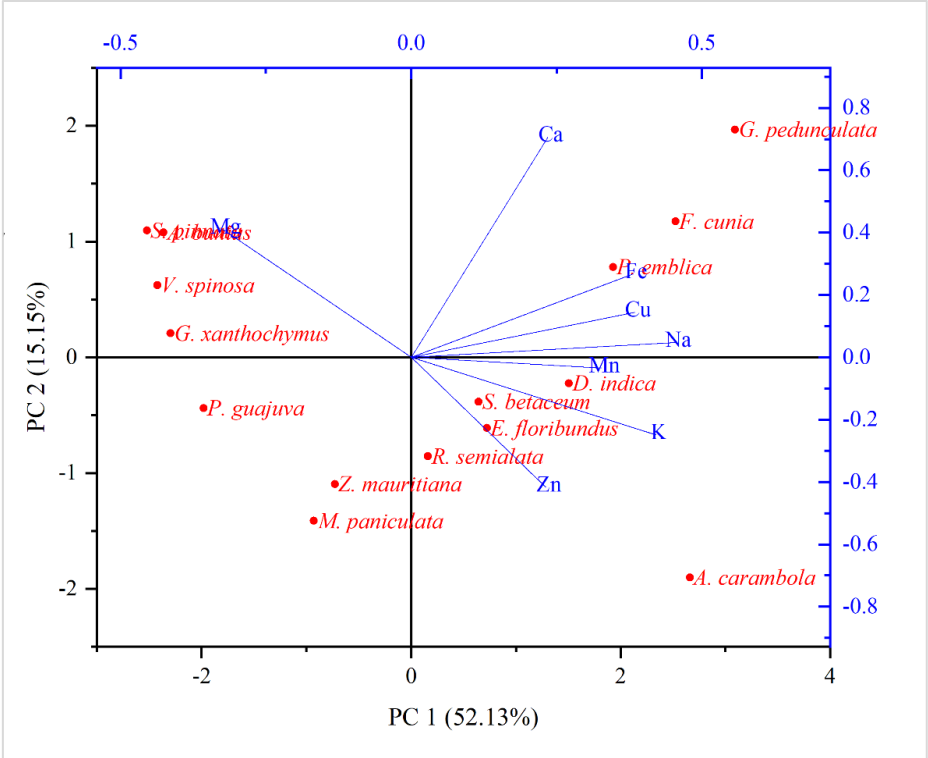

Fig. 5.34 Principal component analysis (PCA) Bi-plot (score and loading plot) of all the mineral elements of fifteen fruit samples.

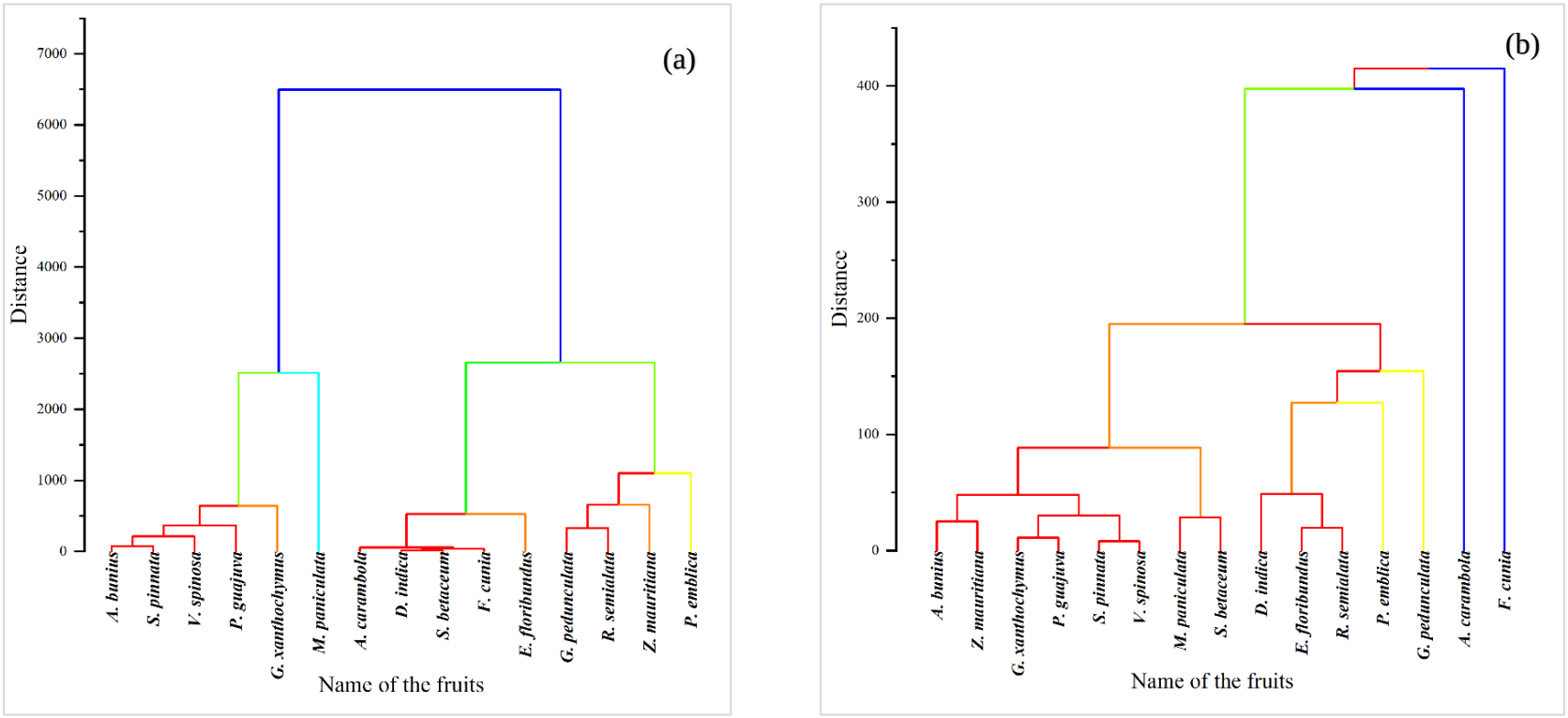

Fig. 5.35 Hierarchical cluster plot based on (a) macro-elements (b) micro-elements.

5.4 DISCUSSION

5.4.1 Physical properties

According to Table 5.2, which presents the physical characteristics of the fifteen fruits studied, the fruits exhibited a range of weights, with some fruits weighing as little as 8.17 grams (*Antidesmus bunius*) and others weighing as much as 650.05 grams (*Dellinia indica*). The circumference of the fruits varied as well, ranging from 0.44 cm (*Rhus semialata*) to 35.56 cm (*Garcinia pedunculata*). Similarly, the lengths of the fruits differed, ranging from 0.30 cm (*Antidesmus bunius*) to 15.60 cm (*Dellinia indica*). The fruits displayed diverse shapes, with some being globose (*Antidesmus bunius, Phyllanthus emblica, Ziziphus mauritiana*), spherical (*Garcinia pedunculata, Psidium guajuva, Spondias pinnata, Vangueria spinosa*), rounded to pear-shaped (*Ficus cunia, Garcinia xanthochymus*), star shaped (*Averrhoa carambola*), round and aggregate (*Dellinia indica*), flattened round (*Rhus semialata*), and oval (*Elaeocarpus floribundus* and *Solanum betaceum*). Additionally, it was observed that weight, circumference, and length of *Averrhoa carambola, Garcinia pedunculata*, and *Rhus semialata* were found to be comparable to those observed in the study conducted by Sharma et al., 2015 [9]. Notably, a critical observation on the shape of the wild edible fruits (WEFs) revealed that most of the 15 studied fruits obtained a globous or spherical shape.

5.4.2 Physico-chemical properties

The moisture content of fruits is a crucial factor in determining their shelf life and quality, as highlighted by Alzamora et al , 2003 [44]. Out of the 15 fruits analysed, only *Rhus semialata* was found to be dry in nature, while the others were having high moisture content. Increasing the moisture content reduces the proximate principles such as protein, fat and carbohydrate, ultimately leading to decreased energy levels [45]. The moisture content varied greatly among the fruits, ranging from 60.79% to 88.04%, which is comparable with the moisture range of minor fruits of Western Ghat

(60.54 -91.45%), however *S. pinnata* (58.80%) of present study showed lower moisture content [46]. *Averrhoa carambola* had the highest moisture content, while *Antidesmus bunius* had the lowest (excluding *Rhus semialata*). *Rhus semialata* is a dry fruit in nature. Eight out of the 15 fruit species studied had above 80% moisture content, which is similar to popular tropical fruits like apples, oranges, mangoes, papayas, lychees, red guavas, and carambolas [47–50]. The percentages of moisture content for *Averrhoa carambola* (88.04%), *Garcinia pedunculata* (86.2%), *Garcinia xanthochymus* (79.43%), *and Rhus semialata* (6.3%) closely matched the findings of Sharma et al., 2015 [9]. In addition, the moisture content in *Solanum betaceum* (82.03%) is almost same with the previous report of 83.56% [51]. On the other hand, the moisture content of *Antidesmus bunius* (60.79%), *Eleaocarpus floribundus* (81.93%), *Phyllanthus emblica* (82.37%), *Spondias pinnata* (58.8%), *and Ziziphus mauritiana* (71.09%) was lower or equal to the report of Khomdram [52]. The moisture content of *Ziziphus mauritiana* was comparatively lower in the current study compared to the work of Sundriyal, 1999 [53]. The remaining fruit samples, including *Dillenia indica* (83.3%), *Ficus cunia* (80.97%), *Microcos paniculata* (72.3%), *Psidium guajuva* (81.63%), *Solanum betaceum* (82.03%), and *Vangueria spinosa* (66.07%) had moisture content similar to some of the wild fruits reported by Sundriyal, 1999 [53]. It is worth noting that the moisture content of *Dillenia indica* was similar as reported by Bhende and Chaitra, 2020 [54], while *Ficus cunia* had nearly the same moisture content as found in the Sikkim Himalayas [53,55]. The moisture content in wild edible fruits of Manipur are also accordance with the indigenous fruits of Sarawak [56]. Similar values were found in moisture content within intra genus like *Antidesma ghaesembilla* (79.1%), *Ficus hispida* (82.84%), *F. racemosa* (81.9%) and *F. carica* (84.6%), and *Dillenia pentagyna* [57]. Comparing the earlier study conducted in Manipur and current study suggested that the moisture content in wild edible fruits decreased over time, likely due to continuous moisture loss by evaporation and respiration [58]. The high moisture content in fruits indicates that they are highly perishable and need to be well-preserved [59].

Total solids refer to the dry matter remaining after removing moisture and is an essential parameter for evaluating fruit quality. It encompasses the non-aqueous

components of the fruit, such as sugars, proteins, fats, and minerals, all of which have a direct impact on the fruit's taste, texture, and nutritional value [50]. In fruits, the relationship between total solids (TS) and moisture content is inverse, indicating that as TS content increases, moisture content decreases and vice versa. Table 2 provides a clear reflection of this relationship, with *Averrhoa carambola* (11.96%) having the highest total solids, while *Antidesmus bunius* (39.21%) had the lowest (excluding *Rhus semialata*). The total solid content of *Rhus semialata* was highest (93.7%) due to natural drying. Fruits with high TS content and low moisture content are generally regarded as higher quality due to their more concentrated flavour and longer shelf life.

The solubility percentage of the fruit samples ranged between 16.67% to 53.33%, with the lowest solubility in *S. betaceum* (16.67%) and highest in *V. spinosa* (53.33%). *Phyllanthus emblica* and *Psidium guajuva* showed the same percentage of solubility in water. Solubility is an important factor in food analysis, as it affects the extraction and separation of compounds from food samples. Many food components, such as pigments, flavour compounds, and nutrients, are often present in small quantities and are distributed unevenly in the food matrix [60,61]. In a study conducted by Chau et al., 2007 [62], solubility percentages were higher than in the current study, ranging from 16.1% to 85.6%, with the highest solubility observed in the fruit of the *Syzygium cumini* tree (85.6%). Similarly, in another study by Madike and Ezeocha, 2013, the solubility of various wild edible fruits ranged from 33.11% to 98.64% [63]. It's important to note that solubility can be influenced by several factors such as the type of solvent used, temperature, and pH [64]. Therefore, the solubility percentages reported in different studies may not be directly comparable. Additionally, the solubility of wild edible fruits can be affected by various factors such as the maturity of the fruit, the processing method, and the growing conditions.

The pH levels of both fresh and dried fruit samples were found to be acidic, with fresh samples generally being more acidic than dried samples, except for *A. carambola* and *Z. mauritiana*. Among the fresh fruit samples tested, pH levels ranged from 2.41 to 5.82, with *G. pedunculata* and *G. xanthochymus* having the most acidic fruits at 2.41 and 2.77, respectively. On the other hand, the pH of the dried samples ranged from

3.09 to 6.98, where *Z. mauritiana* showed the highest acidic fruit and the lowest by *D. indica.* The pH levels of some of the fresh fruits, including *A. bunius, A. carambola, E. floribundus, P. emblica, and S. pinnata* were less acidic, while *Z. mauritiana* was slightly more acidic as compared with the report given by Khomdram [52]. The pH of wild *P. guajuva* (5.82) is higher than the various cultivars of *P. guajuva,* ranging 3.76 to 4.47 [65]. It should be noted that the pH values of wild edible fruits can vary depending on several factors such as the season, maturity stage, and growing conditions [66]. Additionally, the amount of acid in a fruit can be used as an indicator of its maturity stage and can be one of the key measurements for determining its flavour quality and full ripening age for harvesting [67–69] and it may use as indicators in determining its full ripening age for harvesting [70,71]. The pH levels of medium acidic fruits are consistent with some of the important wild edible fruits of Himachal Pradesh [72] and popular commercial fruits such as pineapple, pummelo, apples, oranges, kiwi, grapes, and blueberries [73–76]. It is also worth noting that the pH level of *S. pinnata* was higher than that of *S. mombin* found in Brazil, which had a pH range of 2.27 to 3.36 [77], while *G. xanthochymus* found in Manipur was more acidic than Karnataka, which had a pH of 2.93 [78], but *G. pedunculata* had a higher pH level compared to Assam [79].

The fruit sample exhibited a range of titratable acidity levels, with *Z. mauritiana* displaying the lowest acidity at 0.23%, and *G. xanthochymus* displaying the highest at 4.38%. Titratable acidity is an indicator of the total acidity present in a sample, encompassing both protonated and unprotonated acid molecules [80]. The measured titratable acidity reflects the total acid content of the sample. The titratable acidity values of the examined wild edible species fall within the reported range for other wild edible plants from the Himalayas [81–83]. Notably, the measured titratable acidity of the wild edible fruits was lower compared to previous reports by Sharma et al., 2015, which ranged from 1.35% to 16.51% [9]. However, *A. carambola* exhibited a higher titratable acidity of 1.37%, compared to the sample (0.64%) from Mizoram [84]. It is worth noting that the titratable acidity of *P. guajuva* is comparatively low with the TA of various cultivars of *P. guajuva* [65].

The colour of food is the first criterion consumers use to evaluate its quality. The study examined various fruit samples and measured their colour coordinates (*L**, *a**, and *b**) and indexes (*WI, YI*, and *BI*). *D. indica* had the greater luminosity (*L** = 73.4) and *V. spinosa* had lowest (*L** = 39.54). These *L** values could be correlated with the total pigment (colour) of these families and a higher *L** value in fruits could indicate greater ripeness or freshness, while a lower value could suggest over-ripeness or spoilage [85]. All samples showed positive values for *a** coordinates, indicating redness, and for *b** values, indicating yellowness. *A. carambola* had the maximum *WI* value, while *G. xanthochymus* had the minimum. *G. xanthochymus* had the highest *YI* value, while *V. spinosa* had the lowest. *F. cunia* had the highest *BI* value, while *V. spinosa* had the lowest. Overall, *V. spinosa* had the lowest value, except for *WI*. Evaluating the colour of fruits is a common practice in fruit analysis to determine their ripeness and quality since the colour is a critical sensory quality feature in fresh and processed food products, and it can significantly impact consumer preferences and purchasing decisions. [86,87]. Thus, colour measurement is typically conducted to evaluate the quality of fruits, including their flavour and pigment contents, as it can be correlated with other quality attributes [88,89]. Colour analysis can also provide vital information about the nutritional and health-related components of fruits. These derived indexes also indicate a desirable level of ripeness or freshness in fruits and vegetable [90]. Moreover, colour analysis can assist in controlling fruit processing operations, such as sorting, grading, and packaging, to guarantee product consistency and quality [91]. Several factors can contribute to the variability in fruit colour, such as growth conditions, maturity at harvest, postharvest handling, and environmental conditions during storage [92].

5.4.3 Compositional analysis

A study was conducted to qualitatively investigate the chemical composition of 15 wild edible fruits. Both aqueous and ethanol extracts were examined for various phytoconstituents. The preliminary analysis revealed the presence of several biologically significant compounds including phenolics, flavonoids, tannins, anthocyanins, alkaloids, saponins, cardiac glycosides, steroids, quinones, coumarins,

phlobatannins, proteins, and carbohydrates. Notably, the ethanol extract contained a higher concentration of active components compared to the aqueous extract, consistent with previous findings [93–96].

The proximate analysis assessed the level of ash, crude fat, calorific value, total protein, and total carbohydrate. Ash content recorded highest in *Solanum betaceum* (12.5%) and lowest in *Garcinia xanthochymus* (2%). The ash content in *A. bunius* was higher than the report made by Islary et al. 2017 having 0.51g [97]. The ash content in different wild edible fruits was found to be higher than previously reported, ranging from 2.1% to 4.15% [81]. *Elaeocarpus floribundus* and *Eleaocarpus sikkimensis* had the same ash content at 4%, while *Rhus semialata* was comparable to *Machilus edulis* at 2.65 [81]. The ash content in some other fruits such as *Grewia sapida* [98], *Pyrus pashia*, *Ficus palmata* and *Pyracantha crenulata* [99] was found to be lower. Fruits of *Phyllanthus emblica* also showed higher ash content (3.60%) than previous report from Western Ghat of India having 0.62%, [100]. Fruits of *Ficus cunia* had higher ash content than previous report [55] from Sikkim Himalaya having 0.20%. The amount of ash level in all the fruit samples was higher than the fruits of Sarawak, ranging 0.2% to 4.8% [56]. Fruits of all 15 species showed the value greater than 1.5 g/100g which is not characteristic to most of the commercial fruits. Generally, the ash content in fruits is low, indicating a lower mineral content containing salt of metals and trace minerals [98,101].

Most fruits contain less than 1 gram of fat per serving, and some may not contain any fat at all. However, there are some exceptions such as avocado, which is a high-fat fruit with 15 grams of fat per 100-gram serving [102]. The studied 15 wild edible fruits had total fat content ranged from 0.40% to 11.47%, with the lowest amount of fat in *E. floribundus* and the highest in *R. semialata*. *Fruits such as E. floribundus (0.4%), G. pedunculata* (0.7%), *P. emblica* (0.94), *V. spinosa* (0.72%) and *Z. mauritiana* (0.52%) had fat content less than 1%, which is higher than the report on wild edible fruits of Kani tribes [103]. The fat content of *E. floribundus* differed from that of *Elaeocarpus tectorius*, which had a fat content of 1.13% [100]. *Ficus cunia* (4.34%) had a higher fat content than the earlier report of 0.35% [55]. *R. semialata*

from Manipur had a higher fat content of 11.47% than *R. semialata* from Meghalaya, which had a fat content of 5.82% [104], while the amount of fat present in *R. semialata* is lower than the fruits *Canarium odontophyllum* (26.2%) and *Dacryodes rostrata* (16.1%) [56]. The fat content of wild edible fruits varied in studies conducted by Sundriyal and Sundriyal, 2001 [81], Deshmukh and Waghmode, 2011 [105], Urs et al., 2022 [100], and Rana et al., 2018 [102]. The result revealed that the fat content in wild edible fruits are higher than the values reported by Montville et al., 2013 [106] for some commercial fruits such as grapes (0.47g), mango (0.38g), pineapple (0.12g), and banana (0.33g), and pomegranate (0.95g).

The caloric value of fruit samples was in the ranged of 198.48 kcal/100g to 458.59 kcal/100g with the lowest being *V. spinosa* and the highest being *R. semialata*. The calorific value of *Antidesmus bunius* (289.73 kcal/100g) is found to be comparatively low as reported by Islary et al., 2017 (384.65kcal/100g) [97]. Six out of 15 fruits, including *Averrhoa carambola* (346.57 kcal/100g), *Dellinia indica* (373.08kcal/100g), *Elaeocarpus floribundus* (323.64kcal/100g), *Microcos paniculata* (365.68 kcal/100g), *Psidium guajuva* (333.67kcal/100g), and *Solanum betaceum* (332.96 kcal/100g) were accordance with the caloric value content in wild edible fruits of Western Himalaya [107] and the wild edible of fruits Assam such as *Aporosa dioica* (348.52kcal/100g) and *Ottelia alismoides* (388.50kcal/100g) [108]. The total calorific value of *Averrhoa carambola* (346.57 kcal/100g) was same as *Grewia. sapida* (346.34kcal/100g) [98]. However, *Rhus semialata* had a higher calorific value (458.59 kcal/100g) than the wild edible fruits of Western Himalaya [107] and Meghalaya [104,109]. The range of calorific values of the fruit samples was higher than the indigenous fruits of Sarawak except *Canarium odontophyllum* (339 kcal/100g), which is comparable to *Averrhoa carambola* (346.57kcal/100g). The total calorific value of the fruit samples was much higher than other wild edible fruits reported from different regions of Manipur [52], whereas it is lower than the wild fruits of middle east [110] including *Arbutus pavarii* (790.00kcal/100g), *Nitraria retusa* (49.33kcal/100g) and *Ficus palmata* (565.67kcal/100g). The 15 fruit samples in this study had a high caloric value relative to commonly consumed commercial fruits such as oranges (45 kcal/100g), apples (52 kcal/100g), grapes (57 kcal/100g),

and mangoes (60 kcal/100g) [106], indicating that the wild edible fruits investigated are a good source of energy comparable to most commercially cultivated fruits available in markets.

The present study found a wide range of total carbohydrate values, varying from 8.16% to 36.39%. The highest value was found in *Phyllanthus emblica* followed by *Psidium guajuva* (33.93%), and *Vangueria spinosa* (34.56%) and the lowest concentration was observed in *Antidesmus bunius.* In comparison with the wild edible fruits eaten by Kani tribes, *Antidesmus bunius* had a lower value compared to the intraspecies *Antidesma acidum* (11.57g/100g) and *Antidesmua ghaesembilla* (17.63g/100g). Similarly, *Elaeocarpus floribundus* had 15.68% of total carbohydrate, which is lower than *Elaeocarpus munronii* (23.40g/100g) and *Elaeocarpus serratus* (22.57g/100g). Meanwhile, *Spondias pinnata* had a higher value of 22.24% compared to the *S. pinnata* (15.65g/100g) of the Kani tribe in Agasthyamalai Biosphere Reserve [103]. The carbohydrate content of *Ziziphus mauritiana* (12.48%) was similar to that of *Ziziphus nummularia* (13.52g/100g) and *Ziziphus oenoplia* (11.20g/100g). *Ficus cunia* had 12.49% total carbohydrate, similar to the report given by Dhani et al., 2107 having 16.51%. [55]. However, this value was lower than *Ficus racemosa* (20.47g/100g) [103] and *Ficus palmata* (28.74%) [110]. The estimated range of total carbohydrate in the fruits was lower than that of Western Himalaya (74.29 to 80.71g/100g) [107], Odisha (4.33% to 11.8%) [111], and wild edible fruits of Meghalaya (47.03% to 85.83%) [104,109]. Nonetheless, the carbohydrate content of all the investigated fruits was similar to that of wild edible fruits of Western Ghat [46] and Sikkim [81], except Karonda and Avacado fruit, which had 67% and 0.8% of total carbohydrate, respectively. Notably, *Rhus semialata* had a drastically different total carbohydrate value of 23.08% compared to a previous report of 83.79% [104]. Comparative analysis of the data suggested that the investigated wild edible fruits of Manipur contain reasonable amounts of carbohydrate, comparable to most cultivated fruits, such as apple (13.40%), grape (10.10%), mango (11.80%), orange (10.60%), papaya (9.50%), and pineapple (12.00%) [81].

Estimated protein content of the fruit samples showed that *P. emblica* had the highest protein content (11.66 g/100g) among the 15 samples, followed by *S. pinnata* (7.38 g/100g), while the lowest value was found in *E. floribundus* (0.70 g/100g). The present findings are consistent with Khomdram (2017), [52] that *P. emblica* has the highest protein content (27.14 mg/100g) among 20 fruit samples. *P. emblica* is a popular fruit tree due to its high nutrient content, making it a valuable ingredient in medicinal, culinary, and cosmetic industries [112]. The variation of protein content of *P. emblica* with other fruit species is depicted in Fig. 14. The protein content of *P. emblica* and *S. pinnata* is similar to that of some wild edible fruits of Western Himalaya, ranging 8.47 to 10.49 g/100g [107]. On the other hand, three fruit species had protein level below 1 gram, including *E. floribundus* (0.70 g/100g), *G. pedunculata* (0.86 g/100g), and *R. semialata* (0.75 g/100g), which are comparable with the fruits of *Salacia fruticosa* (0.46 g/100g), and *Syzygium zeylanicum* (0.79 g/100g) [103]. The protein level for *R. semialata* and *G. pedunculata* is almost similar with *G. sapida* (0.78 g/100g) [98] and *O. alismoides* (0.856 g/100g) [108], respectively. Six fruit samples showed protein levels between 1 to 3 g/100g, while four more fruits, including *P. emblica* and *S. pinnata*, had protein level above 3g, which is higher than the cultivated and commercially popular fruits based on the report of USDA in 2013 [106]. The present data on protein value of *A. bunius* (1.10 g/100g) is line with the values of *A. ghaesembilla* (1.17 g/100g), while *Z. mauritiana* (4.50 g/100) showed the higher levels from the previous report of 1.93 g/100g [103]. Comparing the findings of Sundriyal et. al, 2001 [81], it is observed that the present estimates for protein level in wild edible are significantly higher than those of tropical fruits such as apple, grape, banana. fig, mango etc., indicating that the wild edible fruits have the potential to meet protein requirements, and their consumption should be encouraged.

5.4.4 Phytochemical screening

In the quantitative phytochemical analysis, the amount of phenolic compounds, flavonoid, and tannin were estimated. Phytochemicals are naturally occurring bioactive organic compounds found in plants. These bioactive organic compounds

differ vary greatly in structure, mode of action, and biological activities, and are known medicinal properties includes anthelmintic, antioxidant, antimicrobial, and other biological activities [113,114]. Phenolic compounds are recognized to play multiple important biological roles in human health. These compounds have been associated with protective effects against apoptosis, rapid aging, cardiovascular diseases, inflammation, atherosclerosis, and angiogenesis. Plant-derived phenolic compounds, such as flavonoids, phenolic acids, tocopherols, among others, are considered to be natural antioxidants [115,116]. Flavonoids are the secondary metabolites, and they are present in almost all the plants and exhibit varying compositions that are associated with diverse physiological functions. Studies have reported that alkaloids possess antioxidant, anti-inflammatory, anti-cancer, and cardiovascular protective effects. [114,117,118]. Tannins are polyphenolic compounds that are present in various plants and have been found to exhibit multiple biological activities including antioxidant, antimicrobial and cytotoxic activities. Additionally, they are capable of selectively inhibiting the replication of HIV [119,120].

Based on the present observation, it was found that *D. indica* had the highest phenolic content of 5.25 mg GAE/g, followed by *P. emblica* with 5.25 mg GAE/g, and the lowest was observed in *V. spinosa* with 0.9 mg GAE/g. The range of phenolic compounds in the fruit samples was similar to that reported in some wild edible plants, which ranged from 0.87 to 7.02 mg/g [121], and in wild fruits from Romania, which ranged from 184.69 to 727.29 mg GAE/100g [122] The level of phenolic content was relatively low compared to *Pyrus communis* (46.63 mg/g) [123]. *A. bunius* had a lower phenolic content of 1.41 mg GAE/g compared to the reported value of 11.57 mg GAE/g [124]. However, *S. betaceum* had a higher phenolic content of 3.73 mg GAE/g compared to the report of 190 mg GAE/100g [51]. *P. guajuva* also showed a better phenolic level of 2.49 mg GAE/g than the varieties of *P. guajuva* cultivars, ranging from 94.06 to 190.64 mg GAE/100g [65]. The fruits of *F. cunia* had a phenolic content of 4.01 mg GAE/g, which is slightly higher than the *F. cunia* (342.56 mg GAE/100g) of Sikkim Himalaya [55]. The total phenol present in Rubus species of Western Ghat was higher than the present observation in 15 wild edible fruits [125]. Similarly, wild

edible fruits of Burkinia Faso had very high level of TPC ranging 298.5 – 3518 mg GAE/100g [126].Two wild fruits from Assam, *A. dioica* with 146.71 mg GAE/g and *O. alismoides* with 93.86 mg GAE/g [108], contributed to higher phenolic content. Similarly, other wild edible fruits from Assam reported higher levels of phenolic content, ranging from 30.11 to 269.49 mg GAE/g [127]. Fruits of *A. carambola* (2.99 mg GAE/g), *G. pedunculata* (2.17 mg GAE/g), *G xanthochymus* (2.36 mg GAE/g), and *R. semialata* (4.92 mg GAE/g) showed drastically different levels of phenolic content in a previous report from Manipur [9]. Studies revealed that common Indian fruits such as pomegranate (671.1 mg GAE/g), konnow (354.9 mg GAE/g), mango (440.6 mg GAE/g), banana (362.4 mg GAE/g), grapes (538.6 mg GAE/g), and sapodilla (413.9 mg GAE/g) had high level of total phenolics content comparative to most wild edible fruits [128]. The high variation in phenolic content may be due of various environmental factors [129].

The study found that *P. emblica* had the highest flavonoid content at 183.90 mg QE/g, followed by *D. indica* at 94.09 mg QE/g, and *S. pinnata* at 87.74 mg QE/g, while *A. bunius* had the lowest at 3.22 mg QE/g. Flavonoid level in *P. emblica*, *D. indica,* and *S. pinnata* were highly variable compared to the other fruit samples, which ranged from 3.22 to 32.15 mg QE/g. Previous research from Manipur had reported significantly different levels of total flavonoid content in fruits such as *A. carambola*, *G. pedunculata*, *G xanthochymus*, and *R. semialata* ranging from 0.607 to 5.313 mg QE/g [9]. However, the present study revealed higher levels of flavonoid content, indicating greater antioxidant activity. The TFC of *S. pinnata* and *A. carambola* were found to be somewhat comparable with *A dioica* (72.51 mg QE/g) and *O. alismoides* (43.27 mg QE/g), respectively, from Assam [108]. The study of different cultivars of *P. guajuva* [65] confirmed higher levels of TFC (94.33 to 154.19 mg QE/g) than the current observation except *P. emblica*. The cultivar with the lowest value of TFC was similar to that of *D. indica*, but *P. guajuva* had 27.88 mg QE/g, suggesting lesser biological activities. Wild edible fruits were found to have higher TFC levels than traditionally used medicinal plants, which ranged from 0.90 to 14.13 mg/g [130]. *S. betaceum* had 16.16 mg QE/g of TFC, which was higher than the earlier report of 81.22 µg/g [51]. *F. cunia* of Sikkim, Himalaya [55], showed lower levels of

flavonoids than the present study, ranging from 151.46 to 246.70 mg/100g, while a study on different extracts of Rubus fruit species detected high variation in the level of flavonoid content, ranging from 31.53 to 215.00 mg/g [125]. The highest was reported using methanol extract, which was quite higher than the level of *P. emblica* shown in the present study, but comparable with the extract using ethyl acetate at 184.72 mg/g. TFC of wild fruits of Assam [127] ranged from 0.23 mg QE/g to 4.3 mg QE/g, which was comparatively lower than the present observation. In addition, *Polygonum perfoliatum* showed the same amount of TFC with *G. pedunculata* of the current study, which was 4.34 mg QE/g. The wild fruits of Romania [122] and Burkinia Faso [126] also showed lower total flavonoids content. However, common Indian fruits such as pomegranate (473.9 mg QE/g), konnow (261.3 mg QE/g), mango (389.1 mg QE/g), banana (206.1 mg QE/g), grapes (214.5 mg QE/g), and sapodilla (208.3 mg QE/g) showed higher total flavonoids content compared to the present study. The present observation revealed that wild edible fruits, which are often neglected by modern society, contain adequate phenols and flavonoids.

The tannin content of 15 fruit samples was analysed and found to varied between 15.71 to 76.74 mg TAE/g. The lowest tannin content was found in *A. bunius*, while the highest was found in *A. carambola*, followed by *S. pinnata* (67.63 mg TAE/g) and *P. emblica* (54.70 mg TAE/g). *F. cunia* had a tannin content of 34.36 mg TAE/g, which is significantly higher than the *F. cunia* of Sikkim Himalaya with a tannin content of 16.1 mg/100g [55]. A previous study on different extracts of Rubus fruit species showed higher variation in total tannin content (TTC) than the present study, ranging from 7.77 to 628.32 mg/g [125]. However, the TTC in fruit samples are much higher than in *Pteris* species (3.06 – 6.25 mg/g) [131]. *Z. mauritiana* had a TTC of 27.41 mg TAE/g, which is comparable with the intraspecies *Z. lotus* with 31.249 mg/g [132]. The leaves of *P. guajuva* [133] had a much lower tannin content (10.31 – 32.90 µg/mg) than the fruits of *P. guajuva* (44.48 mg TAE/g) in the present study. The investigated wild edible fruits of Manipur were found to contain significant amounts of tannin, comparable to most common fruits of Algeria [134], such as apple, such as apple (5.33 mg TAE/g), apricot (2.26 mg TAE/g), banana (13.65 mg TAE/g), dates (1.77 – 2.25 mg TAE/g), orange (7.5 -10.91 mg TAE/g), peach (1.05 mg TAE/g), and

pear (1.03 mg TAE/g), except pomegranate (34.44 mg TAE/g), which had a similar level of TTC with *F. cunia*. Tannin content in some wild edible fruits of Middle East [110] showed lesser levels, ranging from 1.17 – 3.12 mg/g. Comparison analysis of TTC in investigated wild fruits of Manipur showed better level than the herbs of Manipur [135], such as *Elsholtzia blanda* (17.04 mg/g), *E. communis* (8.72 – 10.87 mg/g), *E. stachyodes* (14.52 mg/g), *Hyptis suaveolens* (15.39 mg/g), *O. Americanum* (9.47 mg/g), *O. basilium* (15.13 mg/g), and *Perilla frutescens* (10.53 mg/g). Similarly, *Aspilia africana*, one of the Nigerian medicinal plants [136] showed lesser TTC at 0.04 mg/100g, while *Bryophyllum pinnatum* (0.51 mg/100g) has similar content with *P. emblica* (54.70 mg TAE/g).

The correlation between total phenolics and flavavoids (R = 0.52) is higher than the report values of Miliauskas et al., 2004 [137], while lower than the report of Lamien-Meda et al., 2008 (R = 0.83) [126]. According to the findings, the fruits analysed contain phytochemicals with reported medicinal and physiological properties that have been traditionally utilized to treat various conditions. A higher concentration of phenolic, and flavonoid compounds corresponded with a stronger antioxidant capacity. Hence, phenolics and flavonoids play crucial roles in reducing the likelihood of developing different human ailments [138].

5.4.5 Antioxidant activity

5.4.5.1 DPPH Assay

The DPPH• (2,2-diphenyl-1-picrylhydrazyl) assay is one of the most widely used and popular methods due to its simplicity, efficiency, low cost, and rapidity [139]. It was developed by Blois in 1985 [140] based on electron transfer and hydrogen atom removal, resulting in a violet solution. The DPPH• free radical is stable at room temperature but is reduced in the presence of an antioxidant molecule, resulting in a colourless solution. This reduction is observed as a decrease in the absorbance of the reaction mixture, and the results can be expressed as either Inhibition % as shown in Fig. 5.22 or IC_{50} values, the concentration of plant extract needed to reduce the

original amount of DPPH• radicals by 50%, as shown in Fig. 5.23 [34,141]. A smaller IC_{50} value corresponds to a higher antioxidant activity of the extract.

From the Fig. 5.22, it is observed that the fruit of *S. pinnata*, which is not commonly used, has demonstrated potential as an antioxidant with activities similar to those of *P. emblica*, Similar observation was made by Dasgupta et al., 2017 [142]. However, *A. bunius* and *V. spinosa* exhibited lowest inhibition percentage.

In a previous study on wild edible fruits of Odisha, it was found that these fruits had good free radical scavenging capacity, as determined by the DPPH assay [143]. Similarly, the current study also revealed a good DPPH radical scavenging activity among the wild fruits investigated. However, there was a wide variation in the IC50 values of DPPH activity, ranging from 78.79 µg/ml in *P. emblica* to 1325.08 µg/ml in *A. bunius*, followed by *G. pedunculata* (242.34 µg/ml). Lower IC_{50} values of DPPH indicate a high level of antioxidant activity, and the fruits with lower IC_{50} values, such as *P. emblica*, *S. pinnata*, *R. semialata*, *F. cunia*, and *P. guajuva*, can scavenge DPPH radicals more effectively to form a stable reduced DPPH molecule. However, the IC_{50} values observed in the present study were lower than those reported for commercial fruits such as grapes (0.79 IC_{50} mg/ml), pineapple (0.83 IC_{50} mg/ml), and guava (1.71 IC_{50} mg/ml) [144], and also from many other common fruits of Algeria [134]. The present study also showed lesser values than those reported for wild edible fruits of eastern Himalaya, ranged from 0.17 µg/ml mg/mL to 0.67 IC_{50} mg/mL [83], and for leaf (9.37 µg/ml) and stem bark (7.36 µg/ml) extracts of *E. floribundus* [145], indicating that the DPPH radical scavenging activity may vary among different plant parts. *P. emblica* from Thailand [146] showed higher scavenging activity than the present study, while *P. emblica* from China [147] showed comparable values with the present study. IC_{50} values for some wild edible fruits of Assam reported in a previous study were higher than the present observation, except for *A. bunius*, indicating higher potential for reducing DPPH radical [127]. Again, the IC_{50} value for *A. bunius* observed quite higher than the previous report of 395.002 µg/ml [97]. The IC_{50} value for *A. carambola* (162.50 µg/mL) and *V. spinosa* (356.08 µg/mL) are comparable with *A. dioica* (168.001 µg/mL) and *O. alismoides* fruit (364.33 µg/mL), respectively

[108]. The IC_{50} value for Rubus fruit species ranging from 11.01 – 352.12 µg/mL were comparable with most of the investigated fruit samples [125].

Pearson correlation coefficients between the IC_{50} values of antioxidant assays and total phenolic, flavonoid and tannin contents were negative, indicating that higher phenolic, flavonoid, and tannin contents are associated with higher antioxidant properties. Flavonoids were found to contribute to the DPPH scavenging activity of *P. emblica* fruit extracts, which is consistent with previous findings [55,83].

5.4.5.2 Reducing Power Assay

In reducing power assay, FRAP (Ferric Reducing Antioxidant Power) and invitro-antioxidant activity assays were executed to evaluate the scavenging activity of investigated fruits. FRAP assay is another method which is widely used to determine the antioxidant properties based on their ability to reduce ferric (III) ions to ferrous (II) ions [148]. This reduction capability is a reflection of the electron-donating capacity of the antioxidants present in the extracts [35]. The antioxidant capacity of the extract is measured by the absorbance of the green to blue Fe^{2+} complex, and the FRAP value, which indicates the scavenging activity, is expressed in terms of the antioxidant capacity. Higher FRAP values indicate stronger scavenging activity.

In this study, the FRAP values obtained ranged from 2.23 mg/g in *G. pedunculata* to 42.57 mg AAE/g in *P. emblica*. The trend in scavenging activity observed in the fruits was consistent with that observed in the DPPH assay. *R. semialata* and *D. indica* demonstrated good activity in both assays, while *G. pedunculata* showing low activity in both. The FRAP reducing power observed in this study was significantly higher than that reported for other wild fruits from eastern Himalaya (3.63 to13.82 mg AAE/g) [83], and central India (0.0518 to 0.111 mg AAE/g) [149]. Similarly, the study also observed good capability of reducing oxidants in comparison to 56 wild edible fruits reported from China [148]. The FRAP values in this study were even higher than those observed in thirteen apple cultivars (71.79 to 137 µmol Trolox/g) [150]. Additionally, similar to our findings, *P. emblica* displayed the greatest FRAP value amongst thirteen fruits of Assam [151], while *A. carambola* also demonstrated

substantial reducing activity in both studies. The present study also showed higher FRAP values than wild edible plants of Assam (64.76 – 799.28 µmol TE/g) [152] and traditional Indian medicinal plants (0.36 – 10.9 mg/g) [130]. The FRAP values of the present study also found to be higher than the Malaysian tropical fruits and vegetables ranging 13.5 – 218 mg AAE/100 g [153,154] and wild edible fruits of Burkinia Faso [126] ranged from 1.21 to 42.35 mmol AAE/100g. Wild edible fruits of Odisha [143], also revealed a good reducing power with FRAP values ranged from 153.09 to 5878.35 µmol AAE/g. The potent activity of the extract could be attributed to the existence of antioxidant compounds in the plants that can interact with free radicals, resulting in the stabilization and cessation of radical chain reactions by providing an electron. In general, the FRAP values obtained reflect all the reductants capable of donating electrons in the sample extracts [35].

The findings of this study suggest that the FRAP assay is significantly correlated with total phenolic content (TPC) ($R = 0.671$) and total flavonoid content (TFC) ($R = 0.748$) at a significance level of $p < 0.05$, and with total tannin content (TTC) ($R = 0.607$) at a significance level of $p < 0.01$. These results suggest that the antioxidant activity of the fruits investigated is largely contributed by TFC (74.8%) compared to TPC (67.1%) and TTC (60.7%). Previous studies have also reported a positive correlation between the FRAP assay and flavonoids [152,155]. Phenolic compounds in plant materials are known to contribute to their overall antioxidant activity primarily due to their redox properties. Several studies have demonstrated a strong correlation between the antioxidant capacity of plant materials and their total phenolic content [156].

The in vitro-antioxidant activity assay is a methodology akin to the FRAP assay, used for evaluating antioxidant activity by reducing ferric (III) ions to ferrous (II) ions with the aid of various reagents. It has been noted that the trend of activity observed through this method is consistent with that of the FRAP assay, as depicted in Figure 28. Similarly, *P. emblica* displayed the highest reducing power, while *G. pedunculata* exhibited the least. However, the range of activity observed through the in vitro-antioxidant activity assay (9.09 – 159.09 mg AAE/g) exceeded that of the FRAP

assay. This implies that the in vitro-antioxidant activity assay is more sensitive than the FRAP assay in detecting the antioxidant activity of the fruits being investigated. It is crucial to note that both methods have their own strengths and limitations, and they should be employed in conjunction to obtain a thorough assessment of the antioxidant activity of a sample.

The in vitro-antioxidant activity assay demonstrated significant correlation with total tannin content at $p < 0.01$ ($R = 1$), and with total flavonoid content at $p < 0.05$ ($R = 0.529$), but not with TPC. Additionally, this assay was positively correlated with the FRAP assay at $p < 0.05$ ($R = 0.607$), while negatively correlated with the DPPH assay at $p < 0.05$ ($R = -0.519$). Several studies have reported a positive correlation between total tannin content and antioxidant activity in various fruits, vegetables, and plants [157–159]. Similarly, in a study on various fruits, including apples, grapes, and strawberries, a positive correlation was observed between antioxidant activity and total tannin content [160]. However, it is important to note that the relationship between total tannin content and antioxidant activity is not always straightforward. For instance, some studies have reported no correlation or even a negative correlation between total tannin content and antioxidant activity [161,162]. This may be attributed to factors such as the type and structure of tannins, the presence of other bioactive compounds that may interact with tannins, and the methods used for measuring antioxidant activity.

5.4.6 Vitamins

Fruits and vegetables are a vital source of vitamins, but concentrations vary among species, cultivars, environmental conditions, and cultural practices [163].

5.4.6.1 Vitamin C (Ascorbic acid)

Vitamin C, also known as L- ascorbic acid, is a water-soluble vitamin. Its solubility in water means that it is not retained in the body for extended periods, in contrast to fat-soluble vitamins. Any excess vitamin C in the body is excreted through urine.

Vitamin C is a necessary co-factor for the synthesis of collagen, L-carnitine, and certain neurotransmitters, as well as playing a crucial role in protein metabolism [164,165]. Additionally, vitamin C also plays a vital role in the body, including in immune system function, and as an antioxidant [166,167]. In addition, vitamin C plays an important role in improving the absorption of nonheme iron, the form of iron present in plant-based foods [168]. A variety of fruits and vegetables contain vitamin C, and it is imperative to maintain sufficient levels of this essential vitamin in the diet [164]. Inadequate vitamin C consumption can lead to scurvy, which is characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility [164,165,168]. The analysis revealed that *P. emblica* has the highest vitamin C content among the fruit samples tested, both in terms of fresh weight (340.85 mg/100g) and dry weight (8.51 mg/100g). On the other hand, *F. cunia* and *G. xanthochymus* had the lowest vitamin C content in terms of fresh weight, at 3.81 mg/100g for both fruits, while *Z. mauritiana* had the lowest vitamin C content in terms of dry weight, at 0.88 mg/100g. Vitamin C is a heat-sensitive nutrient, and drying produce reduces the vitamin C content. The results also indicated that fresh fruits contain a higher level of ascorbic acid than dried fruits, suggesting that fresh fruits are a better source of vitamin C. *P. emblica* is used in Ayurveda, the ancient Indian system of medicine, because of its high constituents of vitamin C, which is effective in scavenging free radical [169]. The size of the fruit is also significant, as the ascorbic acid content in small and large *P. emblica* fruits is 412 mg/100 g and 900 mg/100 g, respectively [170]. Furthermore, three types of *P. emblica* fruit are used as an ingredient of famous ayurvedic tonic chyavanpraash, with varying vitamin C contents (245 mg/100g for big fruit, 275 mg/100g for medium fruit, and 350 mg/100g for small fruit) [171]. The ascorbic acid level found in this study (fresh weight) is comparable to the content of small *P. emblica*. Barthakur and Arnold, 1991 also reported that the consumption of only 10 g (one average-sized fruit) of *P. emblica* would meet the recommended dietary allowance (RDA) for vitamin C [112]. The high content of ascorbic acid in *P. emblica* compared to other wild edible fruits in Manipur is consistent with the report of Khomdram et al., 2014 [172], where they studied nineteen endemic, endogenous fruits of Manipur, and the amount of vitamin C was found to be 375.68 mg/100g. A study of different cultivars of Indian gooseberry revealed a high content of ascorbic acid

ranging 498.81 - 585 mg/100g [173], which is similar to the Indian gooseberry from Bangladesh (509 mg/100g) [174], but higher than the present observation. Ascorbic acid in wild edible fruits of deciduous forests of India ranged from 15.72 to 53.2 mg/100g, with *Z. mauritiana* having 36.01 mg/100g, which is higher than *Z. mauritiana* (9.96 mg/100g) in the present study, but comparable to the earlier report from Manipur, which had 11.9 mg/100g [175]. *A. bunius* (8.21 mg/100g) showed a comparable amount of vitamin C content with the previous report of 7.30 mg/100g [97] and 7.8 mg/100g [175]. *F. cunia* had 3.81 mg/100g of vitamin C, which is lower than that of intraspecies *F. palmata* (37.0 mg/100g), a wild edible fruit from middle east [110]. The ascorbic acid content in *S. betaceum* was 15.25 mg/100g which is lower than the previous report of 33.6 mg/100g [51]. Similarly, *G. xanthochymus* (3.81 mg/100g) and *G. pedunculata* (19.36 mg/100g) showed lower ascorbic acid content than earlier the report from Western Ghat and Northern Himalaya [176]. Apart from *P. emblica*, *P. guajuva* (246.11 mg/100g), *S. pinnata* (207.97 mg/100g), *R. semialata* (134.93 mg/100g), *V. spinosa* (109.71 mg/100g) showed high levels of vitamin C. Yousaf et al., 2021[65] also reported highest value of ascorbic acid in *P. guajuva* cultivars, ranged from 222.26 - 289.43 mg/100 g. In comparison, the fruits of *S. pinnata, R. semialata,* and *V. spinosa* showed much higher levels of ascorbic acid than commonly consumed fruits such as kiwi (75 mg/100g), mango (45 mg/100g), orange (70 mg/100g), papaya (85 mg/100g), strawberries (95 mg/100g), and apple juice (50 mg/100g) [177]. These fruits are abundantly available in the wild. Moreover, these fruits exhibited greater levels of ascorbic acid than commonly consumed fruits in Nigeria [178], such as apple (27.3 mg/100g), guava (69.6 mg/100g), sweet orange (74.67 mg/100g), and pineapple (53.42 mg/100g). Furthermore, *S. pinnata* (86.16 mg/100g) in our study was found to have higher levels of ascorbic acid than *S. pinnata* from other regions in Manipur [172]. Another study on *Spondias mombin*, which is an intraspecies of *S. pinnata*, also reported lower levels of ascorbic acid than the present study [77]. Similarly, *A. carambola* (33.73 mg/100g) had higher levels than the earlier reports from Manipur with 16.38 mg/100g [172], and higher levels than other states in India such as West Bengal (16.12 mg/100g) [179] and Tamil Nadu (16.26 mg/100g) [180]. Therefore, it can be concluded that the *A. carambola* found in Manipur is a better source of ascorbic acid than other states in India. It should also be

noted that Thai wild fruits had lower levels of ascorbic acid than the studied fruits of present observations [178]. The increase in ascorbic acid may be attributed to greater synthesis of glucose (6-) phosphate, a precursor of L-ascorbic acid [181].

5.4.6.2 Vitamin B_1 (Thiamine)

Thiamine, also known as vitamin B_1, is classified as one of the water-soluble B vitamins. It is found naturally in nearly all plants that are commonly used as food and can also be added to certain food products or taken as a dietary supplement. This essential vitamin plays a critical role in energy metabolism, which is vital for the growth, development, and function of cells. It also plays an important role in the proper functioning of the nervous system, muscles, and heart. However, since Vitamin B_1 is water-soluble and somewhat heat-labile, particularly in alkaline solutions, it may be lost during cooking. Thiamine deficiency mainly affects the peripheral nervous system, gastrointestinal tract, and cardiovascular system. It has been demonstrated to be particularly effective in treating conditions such as beriberi, alcoholic neuritis, and the neuritis associated with pregnancy or pellagra. In recent times, thiamine deficiency has been observed primarily in connection with chronic alcoholism, particularly in industrialized nations. This deficiency manifests as the Wernicke-Korsakoff syndrome [182–184]. Based on the analysis conducted, the fruit samples demonstrated varying levels of vitamin B1, ranging from 0.03 to 2.40 mg/100g, with *F. cunia* having the lowest level and *V. spinosa* having the highest. Out of the 15 fruit samples tested, only two samples, *V. spinosa* (2.40 mg/100g) and *S. betaceum* (1.46 mg/100g), exhibited greater content of vitamin B1 > 1 mg/100g, while the rest exhibited < 1 mg/100g. These findings are consistent with previous studies indicating low levels of thiamine in fruits, including common citrus fruits in Bangladesh, which typically have levels between 0.02 - 0.2 mg/100g [174]. A study of Indian Garcinia species revealed thiamine levels of 48 – 52 µg/100g [164], which is lower than the levels found in *G. pedunculata* (0.51 mg/100g) and *G. xanthochymus* (0.59 mg/100g) in the present study. Similarly, a study of five wild edible fruits consumed by the tribal people of north-eastern region in India reported low level of thiamine, ranging 0.05 – 1.1 mg/100g with one fruit (*V. foetidum*) showing no thiamine content [185].

Comparable low levels of thiamine were also observed in 14 native plants of Angola, ranging from 0.08 to 0.75 mg/100g [186], as well as cultivars of apricot fruits, ranging 0.57 – 49 mg/100g [187]. The thiamine level of Pineapple from Goa was found to be 0.079 mg/100g, which is lower than the wild fruits [188]. However, it is important to note that the leaves of 20 plants recorded by Badar and Iliyas in 2015 [189] had higher levels of vitamin B1 compared to the wild edible fruits of Manipur.

5.4.6.3 Vitamin B_2 (Riboflavin)

Vitamin B_2, also known as riboflavin, is a water-soluble vitamin that is not synthesized or stored in the body; therefore, it is crucial to consume riboflavin-rich foods daily. Riboflavin aids body cells in using fat, protein, and carbohydrates from foods to produce energy. It is essential for growth, development, and maintenance of healthy skin, eyes, and nervous system. Riboflavin is a component of various enzyme systems, including the yellow enzyme, and exists as the first riboflavin phosphate (riboflavin mononucleotide) [183,184]. According to the analysis, the range of riboflavin in the fruit samples was found to be higher than that of thiamine, with levels ranging from 0.25 mg/100g to 22.15 mg/100g. The highest level was found in *E. floribundus* and the lowest in *R. semialata*. *P. emblica* showed high riboflavin content (17.85 mg/100g) which contrasts with its thiamine content. Out of the 15 fruits tested, 9 had less than 1 mg/100g of riboflavin, 3 had levels between 1-10 mg/100g, and 2 had levels above 10 mg/100g. These findings are generally consistent with previous studies indicating low levels of riboflavin in fruits, including common citrus fruits in Bangladesh, which typically have levels between 0.01-0.15 mg/100g [174]. However, the present study showed a better range of riboflavin levels. The range of riboflavin for Indian *Garcinia* species was reported to be within 250 - 320 µg/100g [164], which is lower than the levels found in *G. pedunculata* (2.24 mg/100g) and *G. xanthochymus* (5.66 mg/100g) in the present study. However, the present study showed more riboflavin than the five wild edible fruits consumed by the tribal people of north-eastern region in India, ranging from 0.055 – 1.44 mg/100g [185]. *Z. mauritiana* had 1.57 mg/100g of riboflavin, which is comparable to *M. laxiflora* (1.44 mg/100g), while *R. semialata* (0.25 mg/100g) had the same amount of riboflavin as *V. foetidum*

[185]. The levels of thiamine in 14 native plants of Angola, ranged from 0.07 to 6.12 mg/100g [186], which is comparable with some of the wild fruits of Manipur. Similarly, cultivars of apricot fruits found riboflavin levels ranging within 1.56 – 2.56 mg/100g [187]. However, a report on the widely commercialized fruit papaya showed a much lower content of riboflavin at 0.031 mg/100g [188]. It is also worth noting that the vitamin B_2 is not only abundant in fruits, but also in some leaves. It has been reported that leaves of 20 plants exhibited higher levels of vitamin B_2, ranging from 310-400 mg/100g [189].

5.4.7 Minerals

The nutritional value of fruit is greatly influenced by its mineral content. The amount of minerals present in a fruit is dependent on several factors including genetic makeup, climate, soil nutrients, time of harvest, and the location where it is grown [190]. It is reported that, skin and seeds of fruits contribute more mineral content than the flesh of the fruits [191,192]. Minerals play a vital role in the proper development and health of human body, and fruits are generally acceptable as good source of nutrient such as vitamins and minerals [193]. However, an excessive intake of minerals may also have a deleterious effect on the systemic physiology [190]. Minerals are commonly divided into two categories: macronutrients and micronutrients, based on the concentration required for normal tissue function. Macronutrients include potassium (K), calcium (Ca), magnesium (Mg), nitrogen (N) and phosphorus (P), and their concentrations in plant tissues range from 1000 to 15,000 μg/g dry weight. In contrast, micronutrient concentrations are usually 100 to 10,000 times less than those of macronutrients. Mineral micronutrients considered essential for human nutrition include manganese (Mn), copper (Cu), iron (Fe), zinc (Zn), cobalt (Co), sodium (Na), chlorine (Cl), iodine (I), fluorine (F), sulphur (S) and selenium (Se) [194]. In this study, the levels of eight mineral elements were assessed, including four macronutrients (K, Na, Ca, and Mg) (Table 14) and four micronutrients (Fe, Cu, Mn, and Zn) (Table 15). The findings indicate that wild edible fruits contain abundant mineral nutrients. Similar research has shown that wild species are a valuable source of minerals for local communities in different parts of the world [195–198].

5.4.7.1 Sodium (Na)

Sodium is a vital nutrient that plays a significant role in maintaining cellular homeostasis and regulating fluid and electrolyte balance, nerve function, kidney function, and blood pressure [199]. Sodium deficiency is rare and does not occur under normal conditions, even with diets low in sodium [199]. On the other hand, excessive intake of sodium can lead to hypertension, cardiovascular diseases (CVDs), chronic kidney disease, gastric cancer, calcium nephrolithiasis, and osteoporosis [199]. Raw vegetables and fruit juices contain relatively low levels of sodium, ranging from 2.28 to 94.0 mg/100 g and from 0.04 to 277 mg/100 g, respectively[200]. In the analysis, the sodium level in wild edible fruits ranged from 11.56 to 199.27 mg/100g, which is consistent with the findings of Szefer and Grembecka (2006) [200]. *G. pedunculata* contained the highest amount of sodium, followed by *A. carambola* (150.95 mg/100g), *P. emblica* (118.37 mg/100g), and *F. cunia* (117.08 mg/100g), all of which had levels above 100 mg/100g. The remaining fruits had less than 100 mg/100g, with *V. spinosa* having the lowest amount. This observation is higher than the reported values of some commercialized fruits, which ranged from 1 to 16 mg/100g, with most fruits having values within 1-3 mg/100g, including apple, mango, and blueberry [194]. Similarly, citrus fruits in the northern region of Bangladesh had sodium levels ranging from 0.8 to 28 mg/100g [174]. However, estimated sodium content in the 15 wild fruits is comparable to the minor fruits of the Western Ghats of India, ranging from 11.87 to 116.5 mg/100g, with some fruits showing better results [46]. Additionally, six wild edible plants consumed by Bodo of northeast India had sodium level ranging 18.88 – 290.54 mg/100g [106,201]. The level of sodium in *G. pedunculata* (199.27 mg/100g) and *G. xanthochymus* (14.30 mg/100g) was higher than 2.48 mg/100g and 2.06 mg/100g, respectively reported by Parthsarathy and Nandakishore, 2014 [176]. Lower levels of sodium content was observed in some wild green leafy vegetables of North-East India, with levels ranging from 2.7 to 30.7 mg/100g [202]. In contrast, some wild edible fruits of Kolhapur district had higher levels of sodium, ranging 146.3 to 259.6 mg/100g [203] and wild edible fruits of Tripura had levels ranging from 140.67 to 201.26 mg/100g [198]. In summary, the sodium content of wild edible fruits can vary significantly and may be higher than that

of some commercialized fruits, which may have implications for dietary recommendations and health outcomes.

5.4.7.2 Potassium (K)

Potassium is the most abundant mineral element in fruits and vegetable [194,204]. Potassium plays a crucial role in regulating the balance of the body's physical fluid system and facilitating nerve functions by transmitting nerve impulses. Moreover, it is closely linked to heart activity, muscle contraction, and acts as a catalyst in several enzymatic reactions involved in metabolism [205]. Research indicates that a high sodium intake can cause an increase in blood pressure, but an increase in dietary potassium can mitigate this effect. Conversely, a low intake of potassium may raise the risk of developing kidney stones and osteoporosis [206].

Through the analysis of macro-elements, it was observed that the wild edible fruits had a higher potassium content compared to other minerals, with a range of 35.14 to 8783.74 mg/100g. Four wild edible fruits were identified as the richest source of potassium: *A. carambola, D. indica, F. cunia,* and *S. betaceum* with a value of 8783.74 mg/100g. The variation between the highest and lowest of potassium content was highly varied, with the lowest being *G. xanthochymus* with 35.14 mg/100g, which is the only fruit that showed a value lower than 100 mg/100g. However, *G. xanthochymus* had a better level of potassium than the earlier report of 28.4 mg/100g, but nearly comparable to the intraspecies *G. kydia* (38.7 mg/100g) [176].

Out of the 15 fruits, four showed within 100 - 1000 mg/100, such as *A. bunius* (700.36 mg/100g), *P. guajuva* (428.56 mg/100g), *S. pinnata* (666.94 mg/100g), and *V. spinosa* (873.87 mg/100g), while the remaining 10 species showed above 1000 mg/100g, such as *E. floribundus* (8213.92 mg/100g), *G. pedunculata* (5339.99 mg/100g), *M. paniculata* (3048.45 mg/100g), *P. emblica* (6803.94 mg/100g), *R. semialata* (5636.28 mg/100g), including the four co-existing species. The present observed that the potassium level in the 15 wild edible fruits of Manipur was much higher than that of commercialised fruits such as mango (267 mg/100g), orange (179 mg/100g), apple (107 mg/100g), grapes (191 mg/100g), pomegranate (259 mg/100g), kiwi (312

mg/100g), lemon (138 mg/100g), watermelon (112 mg/100g), strawberries (153 mg/100g) etc. [194], except *G. xanthochymus*, which had a lower value than the commercialised fruits. Similarly, the present study also observed higher levels of potassium than the Mexican fruits and vegetables [204] and citrus fruits of northern region of Bangladesh [174].

However, it is noted that the fruit species above 1000 mg/100g of the present study showed comparable amount of potassium with some wild edible plants consumed by Bodo community of northeast India, such as *S. zeylanica* (7684.29 mg/100g), *N. herpetium* (8436.10 mg/100g), *M. perpusilla* (5886.17 mg/100g), *P. chinensis* (1155.28 mg/100g), while two of them showed much higher levels such as *C. hirsuta* (10462.28 mg/100g) and *S. peguensis* (10164.25 mg/100g) [106,201]. However, wild edible fruits of Tripura showed a low amount of potassium with less than 100 mg/100g, ranging from 17.61 to 80.44 mg/100g [198], 49 wild edible fruits consumed by Kani tribe in Agasthyamalai biosphere reserve showed within the ranged of 70-242 mg/100g [103], while commercialised fruits from Timisoara, Romania reported within 83- 210.5 mg/100g [207]. Minor fruits of Western Ghats of India reported levels within the range of 66.91- 642.8 [46] and 20 wild fruits of different region of Manipur also showed levels within the ranged of 60- 650 mg/100g [52]. The differences in genetic traits or other physiological factors may be responsible for these variations. Moreover, according to FAO (2003), the mineral content of fruits can fluctuate up to ten times depending on the location of growth and the time of harvest [44].

Maintaining a Na/K ratio of less than one is important for preventing high blood pressure[208,209]. All the wild fruits analysed in the study have a Na/K ratio less than one, indicating their consumption could help control high blood pressure by reducing the overall Na/K ratio in the body.

5.4.7.3 Magnesium (Mg)

Magnesium is a crucial mineral that is necessary for a diverse range of physiological functions. It plays a significant role in protein synthesis, the release of energy from

muscle storage, and the regulation of body temperature. Proper heart function and bone formation are also reliant on adequate levels of magnesium in the body. Furthermore, magnesium is responsible for activating over 100 enzymes [194,210]. As a key component in the maintenance of human health, magnesium is prominently found in vegetable-based foods. These foods contain Mg^{2+} in the range of 5.5–191 mg/100 g fresh weight. In contrast, a deficiency in magnesium has been linked to the aging process and age-related disorders [190].

The magnesium content of various fruit samples displayed significant variations, ranging from 0.09 mg/100g to 220.74 mg/100g. Among the fifteen fruits studied, *A. bunius* exhibited the highest concentration of magnesium, followed by *S. pinnata* (158.62 mg/100g) and *V. spinosa* (105.95 mg/100g). Fruits of *G. xanthochymus* also exhibited a relatively high concentration of 66.16 mg/100g. Although these fruits displayed higher magnesium levels than certain tropical fruits such as avocado (44 mg/100g) and dates (54 mg/100g), the magnesium concentration of most commercially available fruits is reported to be within the range of 5- 29 mg/100g [194]. The remaining 11 fruits demonstrated lower magnesium levels than these commercial fruits, with the lowest concentration observed in *P. guajuva*. The citrus fruits of the northern region of Bangladesh [174] were also found to possess higher magnesium levels (9.5-49.8 mg/100g) than these 11 wild edible fruits but lower than those of the top four fruits. According to a previous report from the deciduous forests of India [176], it is noted that *G. xanthochymus* (30.62 mg/100g) and *G pedunculala* (35.43 mg/100g) exhibited almost the same level of magnesium, but in the present study, the levels varied significantly, as *G. pedunculata* showed 0.89 mg/100g. The fruit purchased from supermarkets and food markets in Timisoara, Romania [207] also displayed low levels of Mg, ranging from 2.25 to 6.75 mg/100g, which is comparable to some of the fruits in the present study such as *E. floribundus* (2.91 mg/100g) with grapes from Egypt (2.95 mg/100g), *P. emblica* (2.27 mg/100g) with apricot from Greeze (2.25 mg/100g), and *R. semialata* (2.69 mg/100g) with Pear from Bucovat and apples from Italy at 2.8 mg/100g. Similarly, some wild edible plants consumed by Bodo community of northeast India demonstrated magnesium levels within the range of 1.10-9.00 mg/100g [106,201]. However, the wild green leafy

vegetables of northeast India displayed higher levels of Mg, ranging from 34.4 mg/100g to 201.2 mg/100g [202]. Minor fruits of Western Ghats of India reported levels within the range of 4.93 – 95.87 mg/100g [46], which is also higher than that of commercial fruits. The 49 wild edible fruits consumed by Kani tribe in Agasthyamalai biosphere reserve also showed varying levels of Mg, ranging from 3 - 53 mg/100g [103], but with lesser variation than the present study. Moreover, a previous report from Manipur showed varying levels of magnesium in 20 wild fruits, ranging from 3 – 193.25 mg/100g [52], like the present study, where *A. bunius* (193.25 mg/100) exhibited the highest concentration of magnesium.

5.4.7.4 Calcium (Ca)

Calcium is an essential mineral that plays a critical role in maintaining human health by actively participating in various physiological functions of different tissues, such as the musculoskeletal, nervous, and cardiac systems, bones and teeth, and parathyroid gland. Optimal calcium intake is particularly crucial during adolescence to support optimal bone and tooth formation. Conversely, insufficient calcium intake in later adulthood may increase the risk of osteoporosis, a condition that is characterized by weakened bones and decreased bone mass [190,194]. Adequate calcium intake has been associated with several health benefits, such as a reduced incidence of hypertensive disorders during pregnancy, lower blood pressure levels (particularly among young individuals), prevention of osteoporosis and colorectal adenomas, lower cholesterol values, and lower blood pressure in offspring of mothers who consumed sufficient calcium during pregnancy [211]. Therefore, it is vital to ensure sufficient calcium intake during all stages of life for the prevention of osteoporosis and overall well-being.

The investigated fruits exhibited a wide range of calcium concentrations, with values ranging from 16.60 mg/100g to 93.23 mg/100g. *G. pedunculata* had the highest calcium concentration, followed by *P. emblica* (83.57 mg/100g) and *F. cunia* (77.87 mg/100g), while *M. paniculata* had the lowest. The calcium levels in the 15 wild edible fruits of Manipur were comparable to those in the 49 wild edible fruits

consumed by the Kani tribe in the Agasthyamalai biosphere, which ranged from 9 - 77 mg/100g [103]. However, the amount of calcium (37.07 mg/100g) detected in *Z. mauritiana* in the present study was lower than that reported but higher than the report of Mahapatra et al., 2012 (16 mg/100g) [212]. A similar range was also observed in citrus fruits in the northern region of Bangladesh, ranging from 3.1 to 30 mg/100g except lemon (70 mg/100g) [174]. Among the 15 evaluated fruits, the calcium content recorded for 12 species was above 40 mg/100g, which is relatively higher than that of cultivated fruits. This calcium range is comparable to that fruits found in orange (40 mg/100g), figs (35 mg/100g), and kiwi (34 mg/100g), but much higher than calcium content of fruits such as apple (6 mg/100g), banana (5 mg/100g), grapes (10 mg/100g), mango (10 mg/100g), pomegranate (3 mg/100g), and watermelon (7 mg/100g)[194]. *G. xanthochymus* (49.27 mg/100g) and *G pedunculala* (93.23 mg/100g) exhibited higher calcium content than in the previous report of 13.04 mg/100g and 13.21 mg/100g, respectively [176]. However, fruits from Timisoara, Romania [207] displayed comparatively low levels of Ca, ranging from 0.85 to 2.23 mg/100g. Similarly, some wild edible fruits from Kolhapur district displayed even higher levels, ranging from 104.4 mg/100g to 928.4 mg/100g [203]. The minor fruits of Western Ghats of India reported calcium levels within the range of 20.11-66.41 mg/100g [46], which were distributed evenly compared to the fruits in the present study. On the other hand, wild edible plants consumed by Bodo of northeast India demonstrated much lower levels, within the range of 4- 7.41 mg/100g [106,201].

5.4.7.5 Iron (Fe)

Iron (Fe) is the most prevalent trace element in the human body, and iron deficiency anaemia is the most widespread micronutrient deficiency globally. It is a constituent of important compounds such as haemoglobin, myoglobin, ferritin, hemosiderin, and cytochrome enzymes. Haemoglobin, specifically, facilitates the transportation of oxygen to various tissues. Iron deficiency has been associated with immune dysfunction, including impaired cell-mediated immunity, reduced neutrophil, and natural killer cell activity, as well as diminished myeloperoxidase and bactericidal activity, as evidenced by studies conducted on animals and humans. Factors

contributing to iron deficiency include increased physiological requirements, excessive menstrual blood loss, inadequate intake, poor absorption, hookworm infestation, and other infections [213]. Iron is also essential for energy production. Severe iron deficiency leads to hypochromic anaemia. Vegetables and fruits generally have low iron contents, ranging from 0.13 to 3.01 mg/100 g [190]. The recommended dietary allowance (RDA) for iron intake is 8 mg/day for men and postmenopausal women, 11 mg/day for adolescents, 15 mg/day for premenopausal women, and 30 mg/day for pregnant women [214].

Based on the current observations, the iron (Fe) content of the 15 evaluated wild edible fruits ranged from 0.11 to 50.67 mg/100g. There was significant variation in the iron levels among the fruits. The data indicated that *F. cunia* had the highest iron level, followed by *G. pedunculata* (32.58 mg/100g), both of which exceeded 30 mg/100g. The iron level of *G. pedunculata* was comparable to that of *Calamus latifolius* (32 mg/100g) in Manipur [52], but higher than the report by Parthsarathy and Nandakishore, 2014 [176]. Six fruits exhibited iron levels between 10 - 30 mg/100g, including *D. indica* (20.69 mg/100g), *R. semialata* (19.40 mg/100g), *E. floribundus* (18.07 mg/100g), *P. emblica* (15.12 mg/100g), *A. carambola* (12.44 mg/100g), *M. paniculata* (10.40 mg/100g). These levels were comparable to those observed in 16 out of 20 fruits from different regions of Manipur, ranging from 10.85 to 21.55 mg/100g [52]. Furthermore, the iron level of Fe in *E. floribundus* was slightly higher than the previous report from a different region of Manipur (14.25 mg/100g), while *A. carambola* exhibited a slightly lower level than the earlier report of 13.65 mg/100g [52]. Four fruits displayed iron levels between 1- 10 mg/100g namely *A. bunius* (1.57 mg/100g), *G. xanthochymus* (4.12 mg/100g), *P. guajuva* (3.03 mg/100g), and *S. betaceum* (8.79 mg/100g). These levels were comparable to three wild fruits out of 20 from different regions of Manipur [52], although *G. xanthochymus* exhibited a lower iron level compared to the earlier report of 10.82 mg/100g [176]. The 15 minor fruits of Western Ghats of India also reported iron levels within the range of 1- 10 mg/100 [46], with only one fruit exhibiting below 1 mg/100g. Three fruits exhibited iron levels below 1 mg/ml, specifically *S. pinnata* (0.96 mg/100g), *V. spinosa* (0.17 mg/100g), and *Z. mauritiana* (0.11 mg/100g), with *Z. mauritiana* having the lowest iron level.

These values below 1 mg/100g values are comparable to those observed in common commercial fruits which typically range from 0.10 to 0.69 mg/100g [194]. They are also comparable to the iron levels in the 49 fruits consumed by the Kani tribe in the Agasthyamalai biosphere, which ranged from 0.10 to 1.67 mg/100g [103]. Among the 49 species, only eight exceeded 1 mg/100g, and *Z. mauritiana* (0.76 mg/100g) had a higher Fe content than the present report. Similar ranges of iron levels (0.14-1.0 mg/100g) were observed in citrus fruits in Bangladesh [174] and Maxican fruits (0.1 – 3.2 mg/100g) [204]. However, fruits from Timisoara, Romania displayed comparatively low levels of iron [207]. In contrast, some wild edible fruits from Kolhapur district exhibited higher levels, ranging from 15.23 mg/100g to 35.55 mg/100g [203].

5.4.7.6 Copper (Cu)

Copper is an indispensable element in both human and animal physiology, being required only in minute quantities. Functioning as a transition metal, it serves as a vital cofactor for numerous redox enzymes, including catalase, tyrosine, monoamine oxidase, ascorbic acid oxidase, and urease [215]. Additionally, copper facilitates the absorption of iron from the small intestine through a specific transport mechanism. Its involvement extends to neurotransmitter regulation, nutrient metabolism, collagen synthesis, cellular respiration, and immune function. Copper deficiency is uncommon, except in cases of malnutrition, prolonged parenteral nutrition, and malabsorption disorders [213]. Fruits contain small amounts of Cu, ranging from 0.01 to 0.24 mg/100 g. The recommended daily allowance (RDA) of copper ranges between 1.0 and 1.6 mg per day [190].

The analysis of 15 wild edible fruits revealed varying concentrations of copper, ranging from 0.05 to 5.58 mg/100g. Among the fruits examined, the highest copper content was found in *D. indica*, followed by *G. pedunculata* (5.33 mg/100g), and *A. carambola* (2.44 mg/100g). These values significantly surpass the copper content typically observed in cultivated fruits. Conversely, the lowest copper concentration was observed in *M. paniculata*. Five fruit species exhibited copper levels ranging from

0.01 to 0.03 mg/100g, which are compare with the copper content (0.027 to 0.311 mg/100g) found in commonly consumed fruits [194]. A similar range was reported for various citrus fruits in Bangladesh (0.07 to 0.26 mg/100g), with star apple exhibiting a higher content of 0.68 mg/100g [174]. Although star apple exceeded 0.30 mg/100g, its copper content remained lower than that of *A. carambola* (2.44 mg/100g) in the present study. A report stated that fruits purchased from supermarkets and food markets showed copper levels ranging from 0.05 to 0.405 mg/100g, except for pears from Italy, which exhibited lower copper content compared to other fruits [207]. Another study on 15 minor fruits from the Western Ghats of India reported copper levels within the range of 0.02 to 0.91 mg/100g, with *S. pinnata* displaying a copper level of 0.73 mg/100g, higher than the copper content observed in *S. pinnata* (0.13 mg/100g) in the present study [46]. When compared to a previous report from Manipur, some species such as *A. bunius* (1.6 mg/100g), *S. pinnata* (1.15 mg/100g), and *Z. mauritiana* (1.25 mg/100g) exhibited higher copper levels, whereas fruits like *A. carambola* (1.5 mg/100g), *E. floribundus* (1.45 mg/100g), and *P. emblica* (1.25 mg/100g) demonstrated lower copper content [52]. However, certain wild edible fruits from the Kolhapur district exhibited a wider range of copper levels, ranging from 0.43 mg/100g to 7.60 mg/100g [203].

5.4.7.7 Manganese (Mn)

Manganese predominantly resides in the mitochondria and serves as a constituent of numerous crucial metalloenzymes, such as superoxide dismutase, pyruvate carboxylase, arginase, and glycosyltransferase. After absorption in the small intestine, manganese binds to albumin in the bloodstream and is subsequently transported to the liver before being excreted through bile [213]. This element plays a vital role as a component of certain enzymes, particularly those involved in oxygen metabolism. It supports brain function, reproductive processes, blood sugar regulation, and contributes to bone structure. Manganese acts as a co-factor for antioxidant enzymes, including mitochondrial superoxide dismutase [194]. Fruits generally exhibit a relatively low manganese (Mn) content, ranging from 0.01 to 0.66 mg/100g. The recommended daily intake of Mn is 2 mg/day. Occurrences of manganese deficiencies

are exceedingly rare but have been associated with reduced cholesterol levels, abnormalities in red blood cells, and mucopolysaccharide-related issues [190].

The present study revealed that the manganese (Mn) contents in wild edible fruit species of Manipur ranged from 0.12 to 15.79 mg/100g, with four fruits exhibiting Mn levels below 1 mg/100g, comparable to most of the commercially available fruits, ranging 0.011 – 0.927 mg/100g [194]. Similar ranges of Mn levels were also reported in Timisoara, Romania (0.075 - 0.485 mg/100g) [207]; fruits cultivated in Italy (0.621 - 1.55 mg/kg) [216], and fruits and vegetables from Hyderabad (0.0526 - 0.1430 mg/100g) [217]. Among the observed fruit species, manganese content of 5 fruits are within the range of 1 to 5 mg/100g, and another 5 are within the range of 5 to 10 mg/100g. Wild edible fruits from the Kolhapur district exhibited a similar range of Mn levels, ranging from 0.94 to 10.37 mg/100g [203], while one fruit, *P. emblica*, had the highest Mn content exceeding 10 mg/100g, and *P. guajuva* and *V. spinosa* had the lowest Mn levels. A comparison with a previous report from Manipur indicated that *A. bunius* exhibited a much lower Mn level (5.22 mg/100g) compared to the previous value of 21.8 mg/100g, and *S. pinnata* also showed a lower value (0.17 mg/100g) compared to 1.4 mg/100g, whereas *A. carambola* (9.76 mg/100g), *E. floribundus* (5.33 mg/100g), *P. emblica* (15.79 mg/100g), and *Z. mauritiana* (3.48 mg/100g) displayed higher Mn contents [52]. Furthermore, wild edible fruits from Tripura exhibited a wide range of Mn content, from 2.49 to 86.35 mg/100g, with Gamboge showing an extremely high level compared to other fruits from Tripura [198].

5.4.7.8 Zinc (Zn)

Zinc, the second most abundant metal ion in the human body, plays a crucial role in the structure and function of over 100 enzymes. These enzymes include DNA polymerase, RNA polymerase, transfer RNA synthetase, reverse transcriptase, carbonic anhydrase, superoxide dismutase, alcohol dehydrogenase, thymidine kinase, and alkaline phosphatase. Zinc is also involved in the formation of zinc fingers, which play a role in DNA binding, as well as in the structure and function of molecules such as haemoglobin, myoglobin, and cytochromes. Deficiency in zinc is relatively

common and well-documented, and its absence can have detrimental effects on immune system function, as well as impair the senses of taste and smell, and hinder DNA synthesis. Fresh fruits generally contain lower levels of zinc, ranging from 0.02 to 0.61 mg/100 g. The recommended daily allowance (RDA) of zinc is 8 mg/day for females and 11 mg/day for males. Zinc deficiency is commonly observed in individuals with alcoholism, diabetes, malabsorption syndromes, liver and kidney diseases, burns, inflammatory bowel disease, sickle cell disease, and HIV infection. These conditions can significantly increase the risk of zinc deficiency [190,213,218].

The findings of the present study revealed that the zinc (Zn) contents in the wild edible fruit species of Manipur ranged from 0.003 to 39.27 mg/100g, with *A. carambola* exhibiting the highest Zn content followed by *R. semialata* (4.13 mg/100g), while *S. pinnata* displayed the lowest Zn content among the investigated fruits, indicating a significant variation in Zn levels among the fruit species. *A. carambola* stood out with dramatically different Zn content compared to the other 14 fruits, suggesting it as an excellent source of Zn, while *S. pinnata* was shown below 0.01 mg/100g. Five fruits fell within the range of 0.1 to 1 mg/100g, and eight fruits fell within the range of 1 to 5 mg/100g, highlighting most of the investigated wild fruits are a good source of Zn. A comparison with a previous report from Manipur showed that the range of Zn content in wild edible fruits fell within 0.74 to 3.45 mg/100g [52] where the Zn level in *A. carambola* (2.7 mg/100g) was significantly lower compared to the *A. carambola* of the present study. While *S. pinnata* (0.9 mg/100g) exhibited a higher Zn level. Commercially available fruits generally had very low levels of Zn, ranging from 0.04 to 0.68 mg/100g. However, *S. pinnata* in the present study exhibited lower levels compared to fruits like apple (0.04 mg/100g), orange (0.06 mg/100g), papaya (0.07 mg/100g), and avocado (0.68 mg/100g) [194]; Romanian market fruits also exhibited very low levels of Zn, ranging from 0.015 to 0.18 mg/100g [207]. It is worth noting that wild edible fruits from the Kolhapur district exhibited comparable levels of Zn, ranging from 0.94 to 10.37 mg/100g within the range of 1 to 5 mg/100g [203]. While a study of wild edible fruits from Tripura displayed a wide range of Zn content, from 1.11 to 23.7 mg/100g [198], with Gamboge reporting the highest Zn content but it was found to be lower than the value observed in *A. carambola* in the present study. Fruits

cultivated in southern Italy generally showed low levels of Zn content (0.760 - 3.29 mg/kg) [216] compared to the present study, except for *S. pinnata*. Furthermore, fruits and vegetables from Hyderabad exhibited a narrow range of Zn content, ranging from 0.0526 to 0.1430 mg/100g [217].

5.5 CONCLUSION

In this chapter, an analysis was conducted on fifteen different wild edible fruits from Manipur to examine various physico-chemical, phytochemicals, antioxidant activities and mineral elements. The purpose of this study was to gain valuable insights into the composition and potential health benefits of these fruits not to determine the priority among them. The results revealed a diverse range of nutritional parameters including ash, fat, carbohydrate, and protein contents, emphasizing the varied nutrient profiles of the fruits. Furthermore, the fruits exhibited high calorific values, indicating their potential as an excellent source of energy.

The analysis of phytochemical composition revealed varying levels of total phenol, flavonoid, and tannin contents in the fruit samples. Generally, the fruits displayed higher flavonoid and tannin contents compared to total phenol content. Among the samples, *P. emblica* demonstrated the highest phenol content, *M. paniculata* exhibited the highest flavonoid content, and *A. carambola* had the highest tannin content. Phenols, flavonoids, and tannins are renowned for their antioxidant properties and their ability to scavenge free radicals, which are known to contribute to cellular damage and aging. These compounds have been associated with various health benefits, such as anti-inflammatory, anticancer, and cardiovascular protective effects. The variations in phytochemical levels among the fruit samples emphasize the distinctive composition and potential health-promoting properties of each fruit. Incorporating a variety of fruits with diverse phytochemical profiles into one's diet may offer a wide range of bioactive compounds, thereby enhancing overall health and well-being.

The antioxidant scavenging activity of the fruit extracts was assessed using the DPPH method, revealing variations among the different fruits. *P. emblica* exhibited the highest inhibition percentage and the lowest IC50 value, indicating strong antioxidant activity. Conversely, *A. bunius* had the lowest inhibition percentage and the highest IC50 value, suggesting relatively weaker antioxidant properties. Similarly, the FRAP values, which assess the overall antioxidant capacity, indicated that *P. emblica* had the highest value, signifying its potent antioxidant potential. On the other hand, G. pedunculata exhibited the lowest FRAP value among the fruit extracts, indicating comparatively lower antioxidant capacity. These findings underscore the varying antioxidant profiles of the different fruits, with *P. emblica* standing out as particularly rich in antioxidants, while *A. bunius* and *G. pedunculata* demonstrating relatively weaker antioxidant activity.

Furthermore, the analysis of the fruits demonstrated a broad range of vitamin and mineral contents. Fresh samples generally exhibited higher vitamin C content compared to dried samples, with *P. emblica* having the highest vitamin C content in both forms. Various minerals, including potassium, sodium, magnesium, calcium, iron, copper, manganese, and zinc, were observed in the fruits, each exhibiting different levels. These minerals contribute to essential physiological functions in the body.

The nutritional properties of the majority of the wild edible fruits analysed in this study were found to be comparable to, or even better than, those of widely commercialized fruits such as apple, orange, and mango. This suggests that these lesser-known fruits possess significant nutritional value and can be considered as viable alternatives or additions to the commonly consumed fruits.

Overall, the findings highlight the nutritional richness and potential health benefits of these wild edible fruits, which can serve as valuable additions to one's diet by providing essential nutrients, antioxidants, and phytochemicals. Further research and exploration of the potential applications of these fruits in nutrition and health promotion are warranted.

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