CHAPTER VII
PROXIMATE AND MINERAL STUDY

7: Methods

Proximate analysis is the basis for the description of chemical composition and nutritive value of biological materials including food, feeds, faeces, urine, body tissues and fluids (Thimmaiah, 1999). It determines dry matter, moisture, crude fat, crude protein, crude fibre and ash. Proximate analysis of biological food materials is very important to understand the contribution of food staff in health. To analyse the selected medicinal-food plant species in this study standard methods described in Thimmaiah (1999), Raghuramulu et al., (2003). Gopalan et al., (1971), Iswaran (1980) and AOAC (1990) were used.

To study proximate composition fresh samples of the selected species Solanum spirale (leaves, fruit and root), Solanum kurzii (berry), Allium hookeri (whole plant), Pouzolzia hennettiana (shoot), Clerodendrum colebrookianum (shoot), Phoebe cooperiana (fruit), Zanthoxylum rhetsa (shoot) were collected from study sites. After collection, the samples were washed with running tap water followed by rinsing with distilled water and chopping into small pieces. The chopped samples were placed in an electric oven at below 40 °C until a constant weight was attained. The dried samples were ground to powder form to pass through a 1 mm sieve and were stored in sealed in container and kept for further use.

7.1: Determination of Total Carbohydrates

The carbohydrate was estimated by following Anthrone method (Sadasivam and Manikam, 1992) using glucose as a standard. The Anthrone reaction is the basis of a rapid and convenient method for the determination of hexose, aldopentoses and hexuronic acids and either free or present in polysaccharides. Carbohydrates are dehydrates by Conc. H₂SO₄ to form furfural. Furfural de-condenses with acetone to form blue-green coloured complex which is measured calorimetrically at 630 nm.
Reagents:

1. 2.5 N HCL
2. Anthrone reagent: Dissolve 200mg anthrone in 100 ml of ice cold 95% H₂SO₄ (Prepare fresh before use).
3. Standard glucose: for stock, dissolve 100mg in 100ml water, for working standard 10 ml of stock solution diluted to 100 ml with distilled water, store in refrigerator after few drops of toluene.

Method:

1. Weigh 100mg of the sample into a boiling tube.
2. Hydrolyse be keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCL and cool to the room temperature.
3. Neutralize it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100ml and centrifuge.
5. Collect the supernatant and take 0.5 and 1 ml aliquots for analysis.
6. Prepare standard by taking 0, 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard. 0 serves as a blank.
7. Make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water. Then add 4 ml of anthrone reagent.
8. Heat for 8 minutes in a boiling water bath.
9. Cool rapidly and read the green to dark green colour at 630 nm (Lambda-25, UV/VIS spectrometer, Perkin Elmer was used).
10. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
11. From the graph calculate the amount of carbohydrate present in the sample tube by using formula

\[
\text{Sugar value from graph (mg)} \times \frac{\text{Total Vol. of extract (ml)}}{\text{Weight of sample (mg)}} = \text{Amount of carbohydrate (%mg)} \times \frac{\text{Aliquot sample used (ml)}}{\text{X} 100}
\]

Note: Cool the content of all the tubes on ice before adding ice cold anthrone reagent.
7.2: Estimation of crude fibre

Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60% to 80% of the cellulose and 4% to 6% of the lignin. The crude fibre content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incarnated, cooled and weighed again. The loss in weight gives the crude fibre content.

Chemicals:

1. Sulphuric acid solution (0.255 ± 0.005N); 1.25g concentrated sulphuric acid diluted to 100ml. Concentration must be checked by titration.
2. Sodium hydroxide solution (0.313 0 ± 0.005N); 1.25g sodium hydroxide in 100ml distilled water. (Titrate to check concentration)
Procedure

1. Extract 2 g of ground (powdered) material with ether or petroleum ether to remove fat. (Initial boiling temperature 35-38 °C and final temperature 52 °C). If final content is below 1%, extraction may be omitted.

2. After extraction with petroleum ether boil 2 g of dried material with 200ml of sulphuric acid for 30 minutes with bumping chips.

3. Filter through muslin and wash with boiling water until washings are no longer acidic.

4. Boil with 200ml sodium hydroxide solution for 30 minutes.

5. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% H2SO4, three 50 ml portions of water and 25 ml alcohol.

6. Remove the residue and transfer to acidic dish (pre-weigh dish, (W1)).

7. Dry the residue for 2 hours at 130 ± 2 °C. Cool the dish in a desiccator and weigh (W2).

8. Ignite for 30 minutes at 600 ± 15 °C.

9. Cool in a desiccator and reweigh W3

Calculation:

\[
\text{Loss in weigh on ignition} \quad (W2-W1)-(W3-W1)
\]

\[
\% \text{ crude fibre in ground sample} = \frac{\text{Loss in weigh on ignition}}{\text{Weight of sample}} \times 100
\]

7.3: Determination of crude protein

The Kjeldahl method is the standard method of nitrogen determination. The procedure consists of three basic steps:

(i) Digestion of the sample in sulphuric acid with a catalyst that results in the conversion of nitrogen to ammonia.
(ii) Distillation of the ammonia into a trapping solution; and

(iii) Quantification of the ammonia by titration with a standard solution.

In this method nitrogen derived from substitute ammonia like protein is converted into ammonium sulphate and then into ammonia gas. Semi-micro method described in Ishwaran (1980) was followed.

Reagents:

1. Sulphuric acid: (i) concentrated: special grade 1.84; nitrogen free, (ii) 0.01N, accurately standardized.

2. Catalyst mixture: Grind in a mortar 32g potassium sulphate with 5 gram of red mercuric oxide.

3. Sodium hydroxide/ sodium sulphide mixture: Dissolve 400g pure sodium hydroxide in about 700ml distilled water, and dilute to 1 litre. Dissolve 40g sodium sulphide in 111 ml distilled water. Mix the two solutions and filter through glass wool.

4. Mixed indicator: Dissolve 0.1g bromocresol green I 100ml 96% ethanol (solution A). Dissolve 0.1g methyl red in 100ml 96% ethanol (solution B). Mix 100 ml solution A with 20 ml solution B.

5. Boric acid: Dissolve 40g boric acid in distilled water and dilute to 1 litre. Add 40 ml mixed indicator.

Procedure

Weigh into a micro-digestion flask a quantity of product to contain 0.7-1.5mg nitrogen, add 0.5g catalyst mixture and 2.5 ml concentrated sulphuric acid. Commence heating with a small flame until frothing ceases and then heat to boiling. Continue heating until the solution is clear and for 30 minutes longer. Cool and add 8 ml distilled water.

Transfer the solution to the distillation apparatus and rinse the flask with three portions of 2ml distilled water. Add 15 ml of sodium hydroxide/ sodium sulphide mixture and steam distil into 2 ml of boric acid. Collect 10 ml distillate, lower the receiver and collect 10 ml distillate, lower the receiver and distil an additional 2ml. Rinse the outside of the condenser tube and titrate the contents of the flask with 0.01N sulphuric acid. Carry out a blank determination.
The calculation was carried out as:

Let:

Weight (in mg) of sample used: \( W \)

Volume (in ml) of sulphuric acid used in test: \( V_1 \)

Volume (in ml) of sulphuric acid used in blank: \( V_2 \)

Normality of Sulphuric acid: \( N \)

\[
1400 \ (V_1-V_2) \ N
\]

Total nitrogen \( \% = \frac{1400 \ (V_1-V_2) \ N}{W} \)

Crude protein \( \% = \) Total nitrogen \( \times \) factor (6.25).

7.4: Determination of crude fat

The content of fat in a food sample is determined by extracting repeatedly with ether petroleum. The petroleum ether dissolves the fat and hence the amount of fat can be determined by weighing the food stuff before and after ether extraction; the loss in weight gives the fat content in food stuff or evaporating the petroleum ether extract to obtain the fat and then weighing. The fat content of the samples were carried out by following method described in Ishwaran (1980).
Procedure

Crude fat content was determined by extracting 2g of moisture free samples with petroleum ether (bp 40-60 °C) in a Soxhlet extractor; petroleum ether was then evaporated in vacuum evaporator. Increase in weighed of beaker / decrease in weight of sample gave the crude fat.

7.5: Methods for mineral content estimation

Minerals are constituents of skeletal structures, they maintain colloidal state of the body matter and regulate acid base equilibrium, diffusion, osmotic pressure and they are components or an activator of enzymes. Elements regulate an organism in more than one ways.

Sample digestion

10 ml Conc.HNO₃ each was add to one gram each of the sample in digestion flask and kept overnight for digestion. Heat the digestion flask over heater until brown fumes of HNO₃ removed and allowed to cool down. Then add 4 ml perchloric acid to each sample and heated until clear solution is obtained. And the solutions were filtered through Whatman No.1 filter paper. Aliquots were transferred to a 50 ml volumetric flask and make up the final volume to 50 ml with distilled water. Aliquots were used to determine Mn, Cu, Zn, Mg, Na and K by subjected to Atomic absorption spectrophotometer. Iron was determined with colorimetric phenanthroline method and color intensity was measured spectrophotometrically at 510 nm using the UV-Vis spectrophotometer.
7.6: Results:

The proximate compositions of the selected species are given below:

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Moisture (%)</th>
<th>Carbohydrate (%)</th>
<th>Total ash (%)</th>
<th>Crude protein (%)</th>
<th>Crude fibre (%)</th>
<th>Crude fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium hookeri</em> (whole plant)</td>
<td>74.10±0.04</td>
<td>5.30±0.91</td>
<td>6.60±0.27</td>
<td>0.69±0.09</td>
<td>10.64±1.01</td>
<td>0.32±0.02</td>
</tr>
<tr>
<td><em>Solanum spirale</em> (shoot)</td>
<td>76.25±0.093</td>
<td>3.82±0.26</td>
<td>12.54±0.08</td>
<td>0.39±0.08</td>
<td>6.12±1.07</td>
<td>0.37±0.07</td>
</tr>
<tr>
<td><em>Solanum spirale</em> (mature berry)</td>
<td>77.21±1.07</td>
<td>4.09±1.03</td>
<td>13.17±0.25</td>
<td>3.20±1.01</td>
<td>3.04±0.92</td>
<td>0.14±0.91</td>
</tr>
<tr>
<td><em>Pouzolzia bennettiana</em> (shoot)</td>
<td>74.70±1.21</td>
<td>2.76±0.04</td>
<td>10.73±0.84</td>
<td>0.71±1.93</td>
<td>8.03±0.06</td>
<td>0.37±0.04</td>
</tr>
<tr>
<td><em>Phoebe cooperiana</em> (ripen fruit)</td>
<td>67.75±1.04</td>
<td>6.16±1.05</td>
<td>4.49±0.41</td>
<td>3.47±0.96</td>
<td>4.23±0.08</td>
<td>13.08±0.92</td>
</tr>
<tr>
<td><em>Clerodendrum colebrookianum</em> (shoot)</td>
<td>77.90±0.08</td>
<td>4.28±1.08</td>
<td>11.15±0.63</td>
<td>2.36±0.04</td>
<td>4.21±0.05</td>
<td>0.35±0.03</td>
</tr>
<tr>
<td><em>Zanthoxylum rhetsa</em> (shoot)</td>
<td>74.90±1.28</td>
<td>5.43±0.47</td>
<td>12.14±0.51</td>
<td>3.2±0.97</td>
<td>4.05±1.05</td>
<td>0.6±0.07</td>
</tr>
<tr>
<td><em>Solanum kurzii</em> (mature fruit)</td>
<td>74.49±1.16</td>
<td>4.71±0.92</td>
<td>9.62±0.26</td>
<td>5.57±1.06</td>
<td>5.51±0.08</td>
<td>0.76±0.09</td>
</tr>
</tbody>
</table>

Table 7.1: Proximate composition.
Mineral composition

Minerals are constituents of skeletal structures, they maintain colloidal state of the body matter and regulate acid base equilibrium, diffusion, and osmotic pressure and they are components or an activator of enzymes. Elements regulate an organism in more than one ways. The minerals studied from the selected species are given below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of Element (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
</tr>
<tr>
<td><strong>Allium hookeri</strong> (whole plant)</td>
<td>0.485</td>
</tr>
<tr>
<td><strong>Pouzolzia bennettiana</strong> (whole plant)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Clerodendrum colebrookianum</strong> (shoot)</td>
<td>0.215</td>
</tr>
<tr>
<td><strong>Solanum spirale</strong> (shoot)</td>
<td>0.115</td>
</tr>
<tr>
<td><strong>Zanthoxylum rhetsa</strong> (shoot)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Phoebe cooperiana</strong> (fruit)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Solanum spirale</strong> (berry)</td>
<td>0.065</td>
</tr>
<tr>
<td><strong>Solanum kurzii</strong> (berry)</td>
<td>0.175</td>
</tr>
</tbody>
</table>

Table 7.2: Mineral composition.
7.7: Discussion:

Proximate analysis is the basis for the description of chemical composition and nutritive value of biological materials including food, feeds, faeces, urine, body tissues and fluids (Thimmaiah, 1999). It determines dry matter, moisture, crude fat, crude protein, crude fibre and ash. Proximate analysis of biological food materials is very important to understand the contribution of food staff in health. Minerals are constituents of skeletal structures, they maintain colloidal state of the body matter and regulate acid base equilibrium, diffusion, and osmotic pressure and they are components or an activator of enzymes. Elements regulate an organism in more than one ways.

Table 7.1, 7.2, figure 7.3, and 7.4 envisage that Solanum spirale leaf contains 76.25% moisture, 3.82% carbohydrate, 0.39% crude protein, 6.12% crude fibre, 0.37% crude fat. Leaf contains 0.115mg Iron in one gram of sample, 0.07mg of Mn/g, 0.015 Cu mg/g, 0.04mg of Zn/g, 2.25mg of Mg/g, 3.075mg of Na/g of sample and 16.7mg of K/g. The berries contain 77.21% moisture, 4.09% carbohydrate, 13.17% total ash, 3.20% crude protein, 3.04% crude fibre and 0.14% crude fat. 0.06 mg of Fe/g, 0.015mg of Mn/g, 0.012 mg of Cu/g, 0.05mg of Zn/g, 1.25mg Magnesium/g, 2.5mg Na /g and 12.3 mg of potassium per gram sample. From the the above mentioned tables and figures, it may be interpreted that the berry and shoot of S. spirale which are consumed among the tribal folk of the East siang District of Arunachal Pradesh contains considerable proximate and minerals composition that is fit as a healthy food. This medicinal food vegetable is a good source of crude fibre, carbohydrate, magnesium, sodium and potassium that can support proper growth and regulation of body.

Pouzolzia bennettiana contain 74.70% moisture, 2.76% carbohydrate, 10.73% total ash, 0.71% crude protein, 8.03% crude fibre and 0.37% crude fats. Fe content is 0.08mg/g, Zn content is 0.022mg/g, Mg 0.2mg/g, Mn content is 0.04mg/g, Cu content is 8.875mg/g and K content is 22.75/g. Table 7.1, 7.2, and figure 7.5 shows that P. bennettiana contains quite good amount of crude fibre and

This ethnic considerable amount of carbohydrate, crude protein, sodium and potassium. This ethnic food plant is a good source of carbohydrate, protein, fibre, Magnesium, Sodium and food plant is a good source of carbohydrate, protein, fibre, Magnesium, Sodium and Potassium. This medicinal plant contain sufficient proximate and minerals that can easily sustain normal growth and development of rural people who consume this medicinal food as a staple food.
Allium hookeri contain 74.10% moisture, 5.30% carbohydrate, 6.60% total ash, 0.69% crude protein, 10.64% crude fibre and 0.32% crude fat. 0.485mg/g of Fe, 0.03g of Mn/g, 0.017mg of Cu/g, 0.023mg of Zn/g, 1.11mg of Magnesium/g, 3.25mg of Na/g and 9mg of K per gram. Table 7.1, 7.2 and figure 7.2 envisage that A. hookeri contain highest crude fibre among the seven selected medicinal food plant. These data interpretes that this medicinal food plant is a nutraceutical food as adequate concentration of Na and K minerals that regulates cell osmosis, transportation, enzyme activation, sufficient percentage of carbohydrate and fibre may be interpreted this folk food plant as a nutritive food which is fit for human consumption. Accordingly, this plant is a good source of carbohydrate, fibre and potassium with low fats content.

Zanthoxylum rhetsa shoot contain 74.90% moisture, 5.43% carbohydrate, 12.14% total ash, 3.2% crude protein, 4.05% crude fibre and 0.6% crude fat; 0.1mg Fe/g, 0.25mg Mn/g, 0.009mg Cu/g, 0.03g Zn/g, 1.76mg of magnesium/g, 4.3g Na/g and 8.6mg K/g which is given in table 7.1, 7.2 and figure 7.8. Crude protein, crude fibre, magnesim, sodium and potassium are considerably high in this plant that makes it a nutraceutical food. Hence, Z. rhetsa is a good source of proximate and minerals with low fat content.

The proximate composition of Clerodendrum colebrookianum is moisture 77.90%, 4.28%, 11.15% total ash, 2.36% crude protein, 4.21% crude fibre and 0.35% crude fat. The minerals concentration in mg per gram samples are 0.215mg of Fe/g, 0.105mg/g of Mn, 0.0425mg of Cu/g, 0.056mg Zn/g, 2.55mg of Manganes/g, 4.3mg of Na/g and 24.5mg of K per gram of sample. Table 7.1, 7.2 and figure 7.7 reflects that this medicinal food plant as used by the tribal people of the study area is a good source of carbohydrate, crude fibre and minerals.

From the table 7.1, 7.2 and figure 7.6 it can be interprete that Phoebe cooperiana fruit is contains 67.75% moisture, 6.16% carbohydrate, 11.62% total ash, 3.47% crude protein, 4.23% crude fibre and 13.08% crude fat. The mineral content is 0.08mg of Fe in one gram of sample, 0.1mg Mn/g, 0.0055mg of Cu/g, 0.063mg of Zn/g, 1.3 of Mg of K/g of sample. This ethnic food which is also eaten by birds and other wild animals is an intersting food source with an excellent source of fats. The further study on this plant regarding conservation, cultivation etc. should be carried out.
*Solanum spirale* berry contain proximate composition of 74.49% moisture, 4.71% carbohydrate, 9.62% total ash, 5.57% crude protein, 5.51% crude fibre and 0.76% crude fat and mineral composition is 0.175mg Fe/g, 0.03mg Mn/g, 0.01mg Cu/g, 0.135mg Zn/g, 1.68mg magnesium/g, 2.05mg Na/g and 13.4mg K/g. Table 7.1, 7.2 and figure 7.9 envisage that this berry is a good source of carbohydrate, protein, fibre, fats, Iron, Zinc, Magnesium, Sodium and Potassium.

The data of the selected medicinal food plants reflects that these medicinal foods provide sufficient amount of nutrients needed for normal body function, maintenance and reproduction. Except *Phoebe cooperiana* fruit all the studied medicinal foods contain low crude fats, this fact may be the reason that no obese person have been seen in the studied area. They are good source of fibre and fibre can decrease the concentration of high cholesterol level in body. Minerals are very important and essential ingredients of diet required for normal metabolic activities of body tissues. They are important food and highly beneficial for the maintenance of health and prevention of diseases. These medicinal food vegetables are the cheapest source of carbohydrate, fibre, crude fats, crude proteins, vitamins and minerals.

The highest moisture was recorded in *C. colebrookianum* shoot and lowest in *P. cooperiana* fruit; highest carbohydrate was calculated in *P. cooperiana* fruit and lowest in *Z. rhetsa* shoot; highest crude protein was recorded in *S. kurzii* berry and lowest in *S. spirale* leaf, crude fibre was recorded highest in *C. colebrookianum* and lowest in *S. spirale* berry and crude fat was recorded highest in *P. cooperiana* fruit and lowest in *Z. rhetsa* shoot. Fe was recorded highest in *A. hookeri*; Manganese was recorded highest in *Z. rhetsa* shoot and lowest in *S. spirale* berry; Copper was recorded highest in *P. bennettiana* and lowest in *P. bennettiana*; Zinc was recorded highest in *S. spirale* berry and lowest in *P. bennettiana*; Magnesium was recorded highest in *C. colebrookianum* and lowest in *P. bennettiana*; Sodium was recorded highest in *P. bennettiana* and lowest in *S. spirale* leaf; potassium was recorded highest in *C. colebrookianum* and lowest in *A. hookeri*.
Fig 7.2: Proximate composition of *Allium hookeri*

Fig 7.3: Proximate composition of *Solanum spirale* shoot.

Fig 7.4: Proximate composition of *Solanum spirale* berry.
Fig 7.5: Proximate composition of *Pouzolzia bennettiana*.

Fig 7.6: Proximate composition of *Phoebe cooperiana* fruit.

Fig. 7.7: Proximate composition of *C. colebrookianum* shoot.
Fig 7.8: Proximate composition of *Z. rhetsa* shoot.

Fig 7.9: Proximate composition of *Solanum kurzii* berry.