CHAPTER V

5. PHARMACOGNOSTICAL STUDIES

5.1. INTRODUCTION

Medicinal plant materials are authenticated before any further tests are undertaken based on sensory, macroscopic, microscopic, physico-chemical and chromatographic fingerprint characteristics as the first step towards establishing quality and the degree of purity. As macroscopic identity of medicinal plant materials is based on subjective parameters like shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of cut surface, it is often necessary to re-confirm the findings by microscopy. Microscopic inspection of medicinal plant materials is indispensable for broken or powdered materials and if necessary specimens should also be treated with chemical reagents. Any additional useful information such as vein-islets and palisade ratio should also be included in the test procedures for leaves. Physico-chemical examination by determining moisture, ash values, extractive values etc. is another quality determination procedure for considering chemical quality of herbal drugs. Phytochemical examination and HPTLC characterization of extracts are a few other Pharmacopoeial procedures to obtain quality indicating constants.¹

The study of plant drugs from the pharmacognostical stand point would include the study of the habitat, general characters of the plant from which the drug is derived, its place in the botanical system, the organ or the organs of the plant used, their gross, minute structures in the whole and in the powdered conditions and the chemistry of the constituents especially of those which may be used in therapeutics.
The macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials. This should be carried out before any tests are undertaken. Lack of proper standards of medicinal plants may result in the improper usage which in turn will cause damage not only to the individual using it, but also to the respect gained by the well known ancient system of medicine and the entire work on the plant becomes invalid. Thus, in recent years there has been an emphasis on pharmacognostical standardization of medicinal plants of therapeutic potential. So, the present study is undertaken to standardize *Dodonaea viscosa* (L.) Jacq. pharmacognostically which will help in the correct identification of the drug.

5.2. MATERIALS AND METHODS

5.2.1. Anatomical studies

i. **Collection of plant materials**

The fresh healthy plant leaves of *Dodonaea viscosa* Linn. were collected from Alagarkovil Hills, Madurai, TamilNadu, India during the month of August 2010. The plant was identified and authenticated by DR. P. Jayaraman, Botanist, Plant anatomical research centre, Chennai and the voucher specimen number is PARC/2010/2169. The fresh leaves and petiole were also collected and fixed immediately using FAA (Formalin: Acetic acid: 70 % Ethyl alcohol) as fixative agent for anatomical studies. The materials were cut into small pieces before fixing.

ii. **Anatomical studies and staining methods**

**Fixation of plant organ** - The collected materials were cut and left in FAA solution for more than two days.
Dehydration of specimen - After fixing, the specimens were dehydrated with graded series of Tertiary Butyl Alcohol as per the schedule given by Sass, 1940.

Infiltration of the specimens - was carried out by gradual addition of paraffin wax (melting point 58-60º C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections was carried out by customary procedure. The sections were stained with Toluidine blue as per the method published by O’Brien et al. 1964. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin, Fast-green and Iodine in Potassium Iodide (for Starch).

Leaf clearing

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5 % sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s maceration fluid were prepared. Safranin and glycerin mounted temporary preparations were made for macerated / cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and
mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.7

iii. Quantitative microscopy

Quantitative microscopy such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio were observed for the leaf.8

Stomatal Number

Stomatal Number is the average number of stomata per sq.mm of the epidermis of the leaf. For the study of epidermal tissues and stomatal index, the epidermal peelings were prepared by partial maceration technique by employing Jeffery’s maceration fluid. Mechanical pulling by forceps was useful for obtaining peelings.

Stomatal Index

Stomatal Index is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell.
It can be calculated by the formula.

\[ I = \frac{S}{E + S} \times 100 \]

Where

- \( I \) = Stomatal Index
- \( S \) = Number of stomata per unit area
- \( E \) = Epidermal cells in the same area.

**Vein islet number**

Vein islet number is defined as the number of vein islets per square mm of the leaf surface midway between the midrib and margin. The numbers of vein islets were calculated by counting vein islets / mm² from different pieces of lamina of different leaves.

**Vein termination number**

Vein termination number is defined as the number of vein terminations per square mm of the leaf surface midway between the midrib and margin. For the study of vein islet and vein termination number, the leaf material was cut into small bits and boiled in 70 % alcohol for 10 min to remove chlorophyll pigments. Then the bits were immersed in 10 % sodium hydroxide and kept in thermostat at 40°C for 2 to 3 days. The materials were then washed in distilled water, stained with safranin and mounted in glycerin for observation and photography.

**Palisade ratio**

It is the average number of palisade cells beneath each upper epidermal cell of the leaf.
For determining the palisade ratio, the numbers of four epidermal cells are traced and then the palisade cells lying beneath each group are focused and traced. The palisade cells of each group are counted, those being included in the counts which are more than half covered by epidermal cells. The figure obtained was divided by four gives the palisade ratio of that group. The ranges of number of groups from six different fields were recorded.

The above techniques of determination of leaf constants can be used for identification of leaf adulteration.

**Powder microscopic observations**

Leaves were shade dried, powdered, sieved and used for organoleptic and microscopic analysis. The powder was stained with safranin and observed through microscope. Starch grains were observed by staining with iodine in potassium iodide (IKI) and calcium oxalate crystals of the powder were observed under the polarized microscope.

**5.2.2. Physico - Chemical evaluation**

The procedures recommended in Indian Pharmacopoeia and WHO guidelines were followed to evaluate the physico-chemical parameters.9

i. **Ash values**

1. **Total Ash**

The method for determining total ash involves weighing 2-3 g of the sample into tarred silica crucible, which had been previously ignited and cooled before weighing. The sample was incinerated at a temperature not exceeding 450°C and the percentage of total ash with reference to air dried drug was calculated.
2. Water soluble ash

The ash was boiled with 25 ml water for 5 min and filtered through an ashless filter paper. Then the residue was washed with hot water. The filter paper was ignited in a silica crucible at a temperature not exceeding 450°C, cooled and water insoluble ash was weighed. The water soluble ash was calculated by subtracting the water insoluble ash from the total ash.

3. Acid insoluble Ash

Acid insoluble ash was determined by boiling the ash with 25 ml dilute hydrochloric acid for five minutes and filtered through an ashless filter paper. The filter paper was ignited in the silica crucible, cooled in a dessicator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried drug.

4. Sulphated Ash

About 5 gm of the powdered leaf material was taken in a crucible and ignited to char. The residue was moistened with concentrated sulphuric acid (1ml) and ignited at 800°C ± 25°C cooled and weighed. The percentage of sulphated ash was calculated with reference to air dried drug.

ii. Extractive values

Ethanol soluble extractive

5 gm of dried coarse leaf powder of Dodonaea viscosa (L.) was macerated with 100 ml of 90% ethanol in a closed flask for 24 h, shaken frequently during 6 h and allowed to stand for 18 h. Filtered immediately and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 105°C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried drug.
Water soluble extractive

About 5 gm of coarse powder was weighed and macerated with 100 ml of water in a stoppered flask and drops of chloroform was added, shaken frequently during the first 6 h and allowed to stand for 18 h. Then it was filtered, 25 ml of the filtrate was evaporated to dryness in a tarred china dish at 105°C and weighed. The percentage of water soluble extractive was further calculated.

iii. Loss on Drying

About 1 gm of the powdered drug was weighed in a glass stoppered bottle and placed in hot air oven at 105°C until a constant weight was obtained. It was then cooled, weighed and the percentage loss on drying was calculated.

iv. Crude Fibre Content

A quantity of 2 gm of the sample was defatted with ether and boiled with 200 ml of 1.25 % sulphuric acid for 30 min. It was then filtered and washed with boiling water to remove acid. The residue was boiled with 200 ml of 1.25% sodium hydroxide for 30 min. The contents were filtered and the residue was finally washed with water. The residue was dried at 110°C to constant weight and ignited at 600°C for 30 min. The crude fibre content was calculated.

5.2.3. Elemental analysis

Minerals present in medicinal plants are of great importance to understand their pharmacological actions. Minerals play an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living organism which will lead to the formation of active organic constituents. Their deficiency causes diseases, whereas the excess presence may cause toxicity.
Consumption of raw herbal drugs from the medicinal plants contaminated with heavy metals can cause serious consequences on human health. For getting desirable therapeutic benefits, quality of these herbal products must be ensured in terms of metal contamination. Therefore, assessment of heavy metals in medicinal plants is important to control the level of contaminants in herbal raw materials.

Atomic Absorption Spectroscopy (AAS) is used to determine the elements present in the herbs. AAS technique is based on the fact that when atoms, ions or ion complexes of an element in the ground state are atomized in a flame, the absorbed light has the characteristic wavelength of that element. If the absorption process takes place under reproducible conditions the absorption is proportional to the number of absorbing atoms. The concentration of the metal is determined by interpolation of the calibration curve. The instrument used was Perkin Elmer Atomic Absorption Spectrophotometer.

**Procedure**

Three standard solutions of the element to be determined covering the concentration range recommended for the instrument were prepared. Nitric acid and perchloric acid used in the preparation of the substance being examined were also added to the standard solutions in the same concentration. After calibration of the instrument, each standard solution was introduced into the flame three times and the steady reading was recorded. The apparatus was thoroughly washed after each introduction. A calibration curve was prepared by plotting the mean of each group of three readings against concentration. The plant extract prepared above was then introduced into the flame and the reading was recorded. The sequence was then repeated twice. Using the mean of three readings, concentration of the element was
determined from the calibration curve. The process was repeated for the determination of other elements using different lamps.

5.3. RESULTS AND DISCUSSION

5.3.1. Macroscopy

The organoleptic and morphological features of leaf of *Dodonaea viscosa* were observed (Fig. 5.1). Leaves are simple, lanceolate and acute at both ends and narrowed to distinct petiole, stipulate, entire margin, 3.8 – 10 cm x 0.6 - 3.9 cm and symmetrical base. The midrib prominent with closely arranged lateral nerves with pinnately parallel venation. Color is dark green on the upper surface and pale green on the lower surface with no odour and sour taste. Upper surface is shining, more or less viscid with yellowish resinous exudation.

![Fig. 5.1: Morphology of the leaf](image)

(a) Upper surface  (b) Lower surface

*Fig. 5.1: Morphology of the leaf*
5.3.2. Anatomy of leaf

Leaf consists of a thick conical midrib and uniformly thick lamina (Fig. 5.2(1)). The midrib is semicircular and slightly raised on the adaxial side, prominently thick and conical on the abaxial side. It is 450 µm thick (in vertical plane). The abaxial cone is 500 µm wide; the adaxial hump is 400 µm wide.

The epidermis of the adaxial hump is thin; the cells are small and possess conical cuticular spines (Fig.5.2 (2)) the abaxial epidermis is also thin; the cells squarish; some of the cells have spiny cuticle and others have smooth surface. The ground tissue within the adaxial hump consists of about five layers of small collenchyma cells. Also in the abaxial cone these are two or three layers of collenchyma in the subepidermal region. The remaining ground tissue is thin consisting of compact angular parenchyma cells (Fig.5.2 (2)).

The vascular system of the midrib includes an adaxial horizontal band and an abaxial arc of vascular elements (Fig. 5.2(3)). The adaxial system consists of several, parallel compact rows of angular, thick walled xylem elements and transverse of phloem elements. The adaxial vascular strand has a thick arc of sclerenchymatous cap on the outer end of the strand (Fig. 5.2(3)). The abaxial system also consists of a semicircular mass of compact rows of thick walled angular xylem elements and thin arc of phloem elements. Cup shaped arc of sclerenchyma cells occur beneath the abaxial strand (Fig. 5.2(3)).
Lamina

The lamina is dorsiventral. The adaxial epidermis consists of thick cells which are cylindrical with prominent cuticle. At certain places, shallow depression with short, subsessile peltate glandular trichomes are seen (Fig.5.3 (1)). The abaxial epidermis is thin and the cells are thin squarish in shape. The palisade cells are adaxial in position. They form a dense zone of two layers of darkly stained cells (Fig.5.3 (1)).
The spongy mesophyll includes lobed small cells which interlink with each other and form aerenchymatous tissue. The lamina is 170 µm thick.

**Leaf margin**

The marginal part of the leaf is bluntly conical and measures 120 µm thick. The epidermal layer is intact along the margin and the epidermal cells are slightly enlarged and squarish in shape with prominent cuticle. The mesophyll tissues are reduced in size and become more compact (Fig. 5.3 (2)).

**Adaxial epidermis**

The paradermal section of the adaxial epidermis was viewed in surface view. The adaxial epidermal cells are polygonal in outline. The anticlinal walls are thin and straight (Fig. 5.4(1)). The adaxial epidermis is apostomatic.

**Abaxial epidermis**

The abaxial epidermis is stomatiferous and the stomata are diffuse in distribution. The stomata are cyclocytic with the subsidery cells enriching the guard cells. The number of subsidery cells varies from 4 to 6 cells. They are rectangular and thin walled. The guard cells are 70 X 60 µm in size. The stomatal pore is wide elongated and slit like.
Fig. 5.3: Transverse section of *Dodonaea viscosa* leaf
(1) T.S of lamina (40X) and (2) leaf margin(40X)

[AdE- Adaxial Epidermis, GTr - Glandular Trichome, PM- Palisade Mesophyll, SM - Spongy Mesophyll, AbE - Abaxial Epidermis]
The epidermal cells are elongated and rectangular in shape. Their anticlinal walls are thin and straight. (Fig. 5.4 (2))

**Venation pattern of the lamina**

Venation of the lamina was studied from cleared preparation of the leaf. The main veins and major lateral veins are thick. The veinlets are uniformly thin. The veinlets form wide areas of vein islets. The islets are variable in shape and size. The ultimate branchlets of lateral veins give rise to vein terminations which project into vein islets. The vein terminations are unbranched, thick long (or) short, straight or curved (Fig. 5.4(5)) the terminations are mostly unbranched, occasionally they fork into 2 units at the tip (Fig 5.4(4)). Calcium oxalate druses are sparsely seen within the islets (Fig. 5.4(5)).

**5.3.3. Anatomy of Petiole**

The petiole is triangular with two lateral wings and one abaxial wing. It is 600 μm thick and 500 μm wide. The lateral wings are 200 μm long and 100 μm thick. The abaxial wing is 100 μm long and 80 μm thick. The petiole consists of thick squarish epidermal cells with short conical outer tangential walls. Inner to the epidermis occurs a distinct band of palisade cells. The palisade zone is absent in the abaxial part of the petiole (Fig. 5.5). The wings have mesophyll tissues and distinct circular small vascular bundles. The ground tissue of the petiole is parenchymatous, thin walled and compact.

The vascular system consists of a thick hollow, closed cylinder of xylem and phloem. In the outer part of the vascular cylinder occurs phloem. The xylem cylinder consists of short, radial lines of xylem elements and xylem fibres. The xylem
elements are circular and thick walled. The central part of the petiole is occupied by parenchymatous, angular, thin walled, compact parenchyma cells (Fig. 5.5).

Fig. 5.4: Paradermal sections and Venation pattern of lamina
(1) Adaxial epidermis in surface view (10X) (2) Abaxial epidermis in surface view (10X) (3) Stomata (40X) (4) Venation- Low magnification (10X) and (5) Venation- enlarged (40X)

[ AdE - Adaxial Epidermis, AW - Anticlinal Wall, Cr - Crystals, GC - Guard Cell, SA - Stomatal Aperture, SC - Subsidery Cell, St - Stomata, VI - Vein Islets, VT - Vein Terminations]
**Fig. 5.5: Anatomy of petiole**

(1) T.S of petiole(10X), (2) Petiole-enlarged (40X)

[AbW - Abaxial Wing, Ep - Epidermis, GT - Ground Tissue, LW - Lateral Wings, Ph - Phloem, VC - Vascular Cylinder, W - Wing, WB - Wing Bundle, X - Xylem]
5.3.4. Powder microscopy

The powder preparation of the material exhibits the following inclusions:

i. Fibres: Long, narrow thick walled fibres with tapering ends are often seen in the powder. The fibres are 200 µm long and 5 µm thick. The walls are thick and lignified. Wide circular simple pits are seen on the lateral walls of the fibre (Fig. 5.6(1)).

ii. Large spherical masses of druses of calcium oxalate crystals are frequently seen in the powder. The druses are spherical masses of small, triangular individual units aggregated with a spherical body. The druses are 70 µm in diameter (Fig. 5.6(2)).

iii. Epidermal peelings of the lamina: Small pieces of abaxial epidermal peeling are seen in surface view in the powder. The peelings have numerous stomata which are cyclocytic type. The stomata are ensheathed by 5 to 8 rectangular subsidery cells. The stomata are elliptical measuring 10 × 20 µm in size. The cell walls of the epidermal cells are thick and straight (Fig. 5.6(3) & (4)).

iv. Adaxial epidermis: Small peelings of adaxial epidermal part are also seen in the powder. The adaxial epidermis is apostomatic with polygonal cells. The anticlinal walls of the epidermal cells are thick and straight (Fig. 5.7(1) & (2)).
Fig. 5.6: Powder microscopy of the leaf

(1) Fibres (10X) (2) Druses (40X) (3) Stomata (10X) (4) Stomata – enlarged (40X)

[Dr - Druses, EC - Epidermal Cells, Fi - Fibres, SC - Subsidery Cells, St - Stomata]
Fig. 5.7: Powder microscopy of the leaf

(1) Adaxial epidermis (4X) (2) Epidermal cells (10X) (3) Crystals (40X)
(4) Foliar sclereids (40X)

[AdE - Adaxial Epidermis, AW - Anticlinal Wall, Cr - Crystals, Dr – Druses, EC - Epidermal Cells, FScl - Foliar Sclereids, VT - Vein Terminations]
v. Spherical masses of druses are seen diffusely distributed in the vein islets. The druses are aggregation of small units of individual crystals. They are compactly aggregated giving a spiny appearance to the druses (Fig. 5.7(3)).

vi. Foliar sclereids are abundant in the veins of the leaf. The foliar sclereids are elongated and rectangular with thick walls and simple canal like pits. They are mostly attached along the veins or project from the veins into the vein islets. The sclereids are 60 µm long and 10 µm thick (Fig. 5.7(4)).

### 5.3.5. Quantitative microscopy

The observed values for Stomatal number, Stomatal index, Vein-islets number, Vein termination number and Palisade ratio are given in Table 5.1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Number / mm(^2) (Average of 10 fields)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stomatal number (Abaxial)</td>
<td>25.1 - 34.1 - 40.3</td>
</tr>
<tr>
<td>2.</td>
<td>Stomatal index (Abaxial)</td>
<td>5.3 - 8.6 - 9.1</td>
</tr>
<tr>
<td>3.</td>
<td>Vein-islet number</td>
<td>5.7 - 7.2 - 8.3</td>
</tr>
<tr>
<td>4.</td>
<td>Vein termination number</td>
<td>9.8 - 11.1 - 14.5</td>
</tr>
<tr>
<td>5.</td>
<td>Palisade ratio</td>
<td>4.2 - 5.3 - 7.2</td>
</tr>
</tbody>
</table>

Quantitative microscopic data has been highly relied upon pioneer pharmacognosists and are found to be constant for a species. These values are especially useful for identifying different species of genus and also helpful in the determination of genuineness of the plant.
5.3.6. Physico-chemical analysis

Table 5.2: Physico-chemical analysis of *Dodonaea viscosa* leaf powder

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Percentage (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ash Values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total ash</td>
<td>2.52 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Water soluble ash</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Sulphated ash</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>II</td>
<td>Extractive values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water soluble extractive</td>
<td>12.51 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>Alcohol soluble extractive</td>
<td>16.89 ± 1.3</td>
</tr>
<tr>
<td>III</td>
<td>Crude fibre content</td>
<td>2.25 ± 1.51</td>
</tr>
<tr>
<td>IV</td>
<td>Loss on drying</td>
<td>4.56 ± 1.03</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD of triplicates

The physiochemical characterizations of *D.viscosa* leaf are shown in Table 5.2. The physico-chemical parameters are mainly used in judging the purity and quality of the drug. Examination of physico-chemical composition with some Pharmacopoeial analytical tests, which are employed normally for checking quality of herbal drugs as per international standards, is an effective method in evolving quality standards. The results obtained will infer quality in terms of its moisture content, ash content, extractive values which are normally found as standard values for a particular plant. According to WHO, standardization and quality control of herbals are the processes involved in the physicochemical evaluation of crude drug. The constants obtained in the current study will serve as a standard for quality control of *Dodonaea viscosa* in future.
5.3.7. Elemental analysis

The amount of metals present was estimated by Atomic Absorption Spectroscopy and the results are given in Table 5.3.

Table 5.3: Elemental analysis of *Dodonaea viscosa* leaf powder

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Element</th>
<th>Amount</th>
<th>WHO Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lead</td>
<td>4.29 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>2.</td>
<td>Cadmium</td>
<td>0.23 ppm</td>
<td>1 ppm</td>
</tr>
<tr>
<td>3.</td>
<td>Mercury</td>
<td>nil</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>4.</td>
<td>Arsenic</td>
<td>0.03 ppm</td>
<td>5 ppm</td>
</tr>
<tr>
<td>5.</td>
<td>Copper</td>
<td></td>
<td>4.02 mg</td>
</tr>
<tr>
<td>6.</td>
<td>Zinc</td>
<td></td>
<td>3.9577 mg</td>
</tr>
<tr>
<td>7.</td>
<td>Sodium</td>
<td></td>
<td>26.4 mg</td>
</tr>
<tr>
<td>8.</td>
<td>Potassium</td>
<td></td>
<td>156.7 mg</td>
</tr>
<tr>
<td>9.</td>
<td>Selenium</td>
<td></td>
<td>4.98 µg</td>
</tr>
<tr>
<td>10.</td>
<td>Iron</td>
<td></td>
<td>5.8677 mg</td>
</tr>
</tbody>
</table>

Heavy metals such as lead, cadmium, mercury and arsenic were estimated and it was found to be present within the limits of WHO standard. Macroelements (potassium, sodium), microelements (iron, zinc, copper, selenium) were estimated for the leaves and were represented in Table 5.3.
Among the heavy metals, mercury, lead, arsenic and cadmium are toxic and have mutagenic effects even at very low concentration. Several cases of human disease, malfunction and malformation of organs due to metal toxicity have been reported. Along with human beings, animals and plants are also affected by toxic levels of heavy metals. The effects of toxicity vary between metals; for example, while lead poisoning typically may cause abdominal pain, vomiting, severe anemia, hemoglobinuria and the stools have dark color owing to the presence of lead sulfide, mercury poisoning may cause peripheral neuropathy, psychological disturbances and arrhythmias may develop due to the toxic effect of mercury on the myocardium. Late, marked renal impairment occurs due to its nephrotoxic action leading to death.

Some of the trace elements are known to be essential to our body are Cr, As, Co, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, Sn, V, Zn and the other essential major elements are C, H, O, N, S, Ca, P, K, Na, Cl and Mg totaling twenty-six essential elements. Different trace elements in the different medicinal plants will have their definite role for smooth functioning of our body.

Potassium is essential to all organisms with the possible exception of blue green algae. It is a major cation and is important in nerve action which present in intracellular as well as in intercellular fluid. Within the cell it functions as sodium in extracellular fluid by influencing acid base equilibrium, osmotic pressure and water retention. When present in extracellular fluid it influences muscle activities. However, it is moderately toxic to mammals when injected intravenously.

Zinc is essential to all organisms and is an important trace element having a definite role in the metabolism, growth and development. It is an essential component of over 200 enzymes having both catalytic and structural roles. Zinc deficiency is
characterized by recurrent infections, lack of immunity and poor growth. Low intake of zinc may cause coronary artery disease. Clinical studies prove that zinc can have good effect on eliminating ulcer and promoting healing wounds.\textsuperscript{14}

Iron is essential for human body in the production of haemoglobin, in the oxygenation of red blood cells. It is needed for a healthy immune system and for energy production. Severe iron deficiency results in anemia and red blood cells that have a low haemoglobin concentration. In young children, iron deficiency can manifest in behavioural abnormalities including reduced attention, reduced cognitive performance and slow growth. In adults, severe iron deficiency impairs physical work capacity.

Following zinc and iron, copper is the third most abundant trace element in the body. It is an important catalyst for iron absorption. Copper deficiency may be a risk factor for cardiovascular disease, when overt copper deficiency occurs; symptoms include neutropenia, cardiac disorders, osteoporosis and anemia. Excess copper is toxic.\textsuperscript{15}

Selenium is the constituent of glutathione peroxidase and other enzymes reported to have antioxidant property. The deficiency of it may lead to muscular and pancreatic degeneration and haemolysis.

5.4. REFERENCES


