Chapter 5

Pharmacognostic Evaluation of *Meyna laxiflora*
5.1. Introduction and importance of pharmacognostic evaluations

Pharmacognosy is defined as scientific and systemic study of structural, physical, chemical & biological characters of crude drug along with their history, method of cultivation, collection, preparation for market and preservation.\(^1\) It basically deals with the identification controversial species of plants, authentication of commonly used traditional medicinal plants and standardization of natural drugs through morphological, histological, physicochemical and toxicological parameters.\(^2\)

The 75 - 80\% of world population mainly in the developing countries depends on herbal medicine for primary health care. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times.\(^3\) The completion of the rising demand is very difficult, hence to complete this the natural drug is easily adulterated or substituted with low grade material which responsible for the number of toxic reaction out of which some produce lethal effect.\(^2\) These problems can be overcome with the help of pharmacognostic studies which play important role in determining pharmacognostic specifications of medicinal plants. Unlike taxonomic identification, pharmacognostic study includes powder microscopy which helps in identifying adulteration in powder form also because plant loses its morphological identity and can be easily adulterated after drying and converting into powder. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.\(^4\) WHO traditional medicine strategy: 2014-2023 also give stress on strengthening quality assurance, safety, proper use and effectiveness of traditional and complementary medicine to ensure safety of human beings.\(^5\)
5.2. Methodology

5.2.1. Plant material collection and authentication

The Plant *Meyna laxiflora* was collected from Satpuda hills Devgoi, Akkalkuwa, Dist: Nandurbar, Maharashtra, India, in June 2012 and authenticated by Dr. M. B. Patil, HOD, Department of Botany, J.E.S. Arts, Science and Commerce College, Nandurbar by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number QMA-01). (Annexure II)

5.2.2. Morphological evaluation

Macroscopic study was carried out by means of sense organs. Which involve the evaluation of drug the process included the observation of the color, odor, taste, size, shape and texture of the leaf, stem, bark, root and fruit drugs.6

5.2.3. Microscopic evaluation

5.2.3.1. Qualitative microscopic evaluation

Thin transverse section of leaf, stem, bark, root and fruit was taken, dehydrated with different grades of alcohol, stained with phloroglucinol-HCl, concentrated \( \text{H}_2\text{SO}_4 \), and iodine solution and observed under 10X and 45X. The transverse sections were studied. The microscopic powder characteristics of the leaf, stem, bark, root and fruit were performed.7,8

5.2.3.2. Quantitative microscopic evaluation

Quantitative microscopy of the leaf involved determination of stomatal number and index; palisade ratio, vein-islets and vein termination number.7,8

❖ Determination of stomatal number

Stomatal number is the average number of stomata per square mm of the epidermis of the leaf. For determination of stomatal number middle portion of leaf was
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*Meyna laxiflora* Robyns. (Rubiaceae)

cleared by boiling with chloral hydrate solution. The upper and lower epidermis was peeled out separately and mounted in glycerin water. The square of 1mm was prepared on drawing board with the help of stage micrometer and camera lucida. The stage micrometer was replaced by prepared slide and stomata and epidermal cells were traced. The numbers of stomata lying in the area of 1 sq. mm were counted including the cell if at least half of its area lying within the square and average no of stomata was calculated by tracing ten different fields of 1 sq.mm.

 Determination of stomatal index

Stomatal index is the percentage which the numbers of stomata form to the total number of epidermal cells, each stomata being counted as one cell. For determination of stomatal index middle portion of leaf was cleared by boiling with chloral hydrate solution. The upper and lower epidermis was peeled out separately and mounted in glycerin water. The square of 1mm was prepared on drawing board with the help of stage micrometer and camera lucida. The stage micrometer was replaced by prepared slide and stomata and epidermal cells were traced. The numbers of stomata lying in the area of 1 sq. mm were counted including the cell if at least half of its area lying within the square and no of stomata and epidermal cells were counted by tracing ten different fields of 1 sq.mm. Stomatal index was determined by following formula.

\[
\text{Stomatal Index} = \frac{\text{No of Stamata}}{\text{(No of Stamata + No of Epidermal cells)}} \times 100
\]

 Determination of vein-islet numbers

A vein-islet is the small area of green tissue surrounded by the vein-lets. The vein-islet number is the average number of vein-islet per square millimeter of leaf surface. For determination of vein-islet number middle portion of leaf was cleared by boiling with chloral hydrate solution. The square of 1mm was prepared on drawing board with the help of stage micrometer and camera lucida. The stage micrometer was
replaced by cleared leaf slide and veins were traced. The number of vein-islets were counted by considering incomplete vein-islet on any two adjacent sides and excluded on other two sides. The average numbers of vein-islets were calculated by tracing four adjacent fields of 1 sq.mm.

**Determination of vein-termination numbers**

The vein-termination number is defined as the number of vein-let termination per square millimeter of the leaf surface, midway between midrib of the leaf and its margin. For determination of vein-termination number middle portion of leaf was cleared by boiling with chloral hydrate solution. The square of 1mm was prepared on drawing board with the help of stage micrometer and camera lucida. The stage micrometer was replaced by cleared leaf slide and veins were traced. The average number of vein-terminations was calculated by tracing four adjacent fields.

**Determination of palisade ratio**

The palisade ratio is the average number of palisade cells beneath on epidermal cells of a leaf. For determination of palisade ratio middle portion of leaf was cleared by boiling with chloral hydrate solution. Four adjacent epidermal cells and palisade cells beneath epidermal cells were traced with the help of camera lucida. The numbers of palisade cells beneath epidermal cells were counted by including palisade cell in the count with more than half area covered by epidermal cell. The palisade ratio calculated by repeating procedure three times on different parts of leaf.

**5.2.4. Evaluation of physical parameter**

In Physical parameter foreign organic matter, loss on drying, ash value, Total ash, sulphated ash, acid insoluble ash, and extractive value were determined for leaf, stem, bark, root and fruit drugs as per standard method.6,7,9
5.2.4.1. Determination of foreign organic matter

Foreign organic matter means the material which is not collected from the original plant source, part of organ other than mentioned, Insects, moulds or the animal contamination. For determination of foreign organic matter 5 gm of air dried coarsely powdered drug was spread in a thin layer. The sample was inspected with the unaided eye. The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined from the weight of the drug taken.

5.2.4.2. Determination of loss on drying

Loss on drying is the amount of both water and volatile mater which evaporates during drying. For determination loss on drying accurately weighed flat and thin porcelain dish was dried and 2g of sample was transferred, the weight was taken and sample was distributed evenly. Then loaded porcelain dish was kept in oven at 100° C. The sample was dried to constant weight. After drying it was collected to room temperature in desiccator. Weighed and calculated loss on drying in terms of percent w/w.

5.2.4.3. Determination of ash value

Ash is the residue remains after incineration. Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes inorganic variables like calcium oxalate, silica and carbonate content of the crude drug affects total ash value. Such variables are then removed by treating with acid and then acid insoluble ash value is determined.
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**Determination of total ash**

Accurately weighed 2gm of the air-dried crude drug was taken in a tarred silica dish and incinerated at a temperature not exceeding 450 °C until free from carbon, cooled in a desiccator and weight was taken. The process was repeated till constant weight was obtained. The percentage of ash was calculated with reference to air-dried drug.

**Determination of water soluble ash**

The ash, obtained as per the method described above boiled for 5 minutes with 25 ml of water, filtered, and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

**Determination of acid insoluble ash**

The ash obtained as per method described above and boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, filtered, and collected the insoluble matter on an ash less filter paper, washed with hot water, ignited, and cooled in a desiccator and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

**Determination of sulphated ash**

Silica crucible was heated to redness for 10 minutes and allowed to cool in desiccator and weighed. 2 gm of air-dried drug was weighed and ignited gently until the substance was charred cool. The residue was moistened with 1 ml sulphuric acid. It was heated gently until the white fumes no longer evolved and ignited at 800°C ± 25°C until all black particles had disappeared. Ignition was conducted in place protected
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from air currents. Crucible was cooled and few drops of sulphuric acid were added and ignited. Then it was allowed to cool and weighed.

### 5.2.4.4. Determination of extractive value

The total soluble constituents of the drug in any particular solvent or mixture of solvents may be called as extractive value. Different extractive values like water soluble extractive, alcohol soluble extractive, chloroform soluble extractive and petroleum ether-soluble extractive value were determined by standard method.

- **Determination of water soluble extractive value**

  5 gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs.

- **Determination of alcohol soluble extractive value**

  5 gm of air dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs.
Determination of chloroform soluble extractive value

5 gm of air-dried coarsely powdered drug was macerated with 100 ml of chloroform of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of chloroform, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of chloroform soluble extractive value was calculated with reference to air-dried drugs.

Determination of petroleum ether soluble extractive value

5 gm of air-dried coarsely powdered drug was macerated with 100 ml of petroleum ether of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of petroleum ether, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of petroleum ether soluble extractive value was calculated with reference to air-dried drugs.

5.3. Results and Discussion

5.3.1. Macroscopic characters

Leaves are ovate-oblong arranged oppositely or whorls of three. Strong spines are bent at an acute angle, shining with smooth texture. The length of the leaf is between 4.0 to 15 cm and the breadth is between 1.5 to 9 cm. Petioles are comprise between 1.0 to 3.0 cm. Leaves have characteristics odor and taste. (Figure 5.1)

Stem having different shape, young stem commonly polygonal while matured are rounded. Nodes and internodes are absent. Stem is dark brown in color with long spines comprising ranging from 1 to 3 cm. Odor and taste are characteristic with rough fracture. (Figure 5.2)
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**Bark** gets quilled shape after drying. The outer surface is dark brown while inner light brown in color, having longitudinally striated. The fracture is short and rough. Odor and taste are characteristic. (Figure 5.3)

**Root** has very long, cylindrical roots with branched, tough, dark-brown externally, bark thin, outer surface rough, with fracture hard, characteristic odor and taste.(Figure 5.4)

**Fruits** were nearly globular, fleshy, smooth, and purplish when ripe and green at early stages with characteristic odor and slightly bitter taste.(Figure 5.5)

### Table 5.1. Macroscopic characters of *Meyna laxiflora*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Leaf</th>
<th>Stem</th>
<th>Bark</th>
<th>Root</th>
<th>Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>Green</td>
<td>Dark brown</td>
<td>dark brown (outer surface)</td>
<td>dark brown</td>
<td>purplish when ripe and green at early stages</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>light brown (inner surface)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Odor</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Slightly bitter</td>
</tr>
<tr>
<td>4</td>
<td>Size</td>
<td>Length 4. to 15 cm and breadth 1.5 to 9 cm</td>
<td>Varying</td>
<td>Varying depend upon collection</td>
<td>Varying</td>
<td>Varying</td>
</tr>
<tr>
<td>5</td>
<td>Shape</td>
<td>Ovate-oblong</td>
<td>Polygonal (Young) Rounded (Mature)</td>
<td>Curved after drying</td>
<td>Cylindrical</td>
<td>Globular</td>
</tr>
</tbody>
</table>
5.3.2. Microscopic evaluation

5.3.2.1. Qualitative microscopic evaluation

❖ T. S. of leaf

The upper and lower epidermis consists of compact cells with cuticle. Paracytic stomata available at lower surface of the leaf. The mesophyll tissue consists of upper palisade cells and few layers of lower spongy cells. Collenchymas available at below upper and lower epidermis, midrib contain vascular bundle in which xylem is surrounded with phloem. Starch grains and calcium oxalate crystals are also found. (Figure 5.6)

❖ T.S. of stem

The cork is 2-3 celled thick, cells are rectangular in shape. Epidermis is uniseriate and covered with thin cuticle. The epidermal cell is rectangular in shape. Periderm consists of many layers; the cortex consists of collenchymas and parenchyma. Endodermis is made up of one layer. Medullary ray is present. Pericycle comprises of polygonal cells and small cells of parenchyma. The vascular bundle is collateral and open. The phloem consists of many layers of compact parenchyma cells, sieve tube cells and companion cells. The xylem is lignified and thick-walled vessels available in rows with varying size. (Figure 5.7)

❖ T.S. of stem bark

The cork is 2-3 celled thick, cells are rectangular in shape. The cortex consists of collenchymas and parenchyma with calcium oxalate crystals and starch grain cell. The phloem consists of many layers of compact parenchyma cells, sieve tube cells and companion cells. The xylem is lignified and thick-walled vessels available in rows with varying size. (Figure 5.8)
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❖ T.S. of root

The cork is narrow with dark brown color. Phelloderm consists of elongated cell. Cortex composed of many layers which contain calcium oxalate crystals and starch grain cell. Phloem consist several sieve tissue fixed in parenchymatous cell. Xylem is lignified and consists of tracheidal vessels. (Figure 5.9)

❖ T.S. of fruit

The fruit is circular in cross sectioned view showing epidermis, embryo, pericarp, placental tissue, vascular bundle and calcium oxalate crystals. (Figure 5.10)

❖ Powder microscopy

Powder microscopy of leaf showed paracytic stomata, palisade cells, xylem vessels, phloem fiber, starch grains and calcium oxalate crystals. (Figure 5.11) Powder microscopy of stem showed thick portion of cork, parenchyma, phloem fiber, starch grains and calcium oxalate crystals. (Figure 5.12) Powder microscopy of Stem bark showed thick portion of cork, phloem fiber, starch grains and calcium oxalate crystals. (Figure 5.13) Powder microscopy of root showed cork cell, parenchyma, xylem vessel, phloem fiber, starch grains and calcium oxalate crystals. (Figure 5.14) Powder microscopy of fruit showed xylem vessels and calcium oxalate crystals. (Figure 5.15)
5.3.2.2. Quantitative microscopy

The results of stomatal number and index, palisade ratio, vein-islet number and vein termination number are given in table 5.2.

Table 5.2. Quantitative microscopy of Meyna laxiflora

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stomatal number</td>
<td>16 per mm²</td>
</tr>
<tr>
<td>2</td>
<td>Stomatal index</td>
<td>22 per mm²</td>
</tr>
<tr>
<td>3</td>
<td>Palisade ratio</td>
<td>10 per cell</td>
</tr>
<tr>
<td>4</td>
<td>Vein-islet number</td>
<td>39 per mm²</td>
</tr>
<tr>
<td>5</td>
<td>Vein-termination Number</td>
<td>26 per mm²</td>
</tr>
</tbody>
</table>

Figure 5.1. Morphology of Meyna laxiflora leaf

Figure 5.2. Morphology of Meyna laxiflora stem
Pharmacognostic Evaluation of *Meyna laxiflora*

Figure 5.3. Morphology of *Meyna laxiflora* stem bark

Figure 5.4. Morphology of *Meyna laxiflora* root

Figure 5.5. Morphology of *Meyna laxiflora* fruit

Figure 5.6. T.S of *Meyna laxiflora* leaf

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Figure 5.7. T.S of *Meyna laxiflora* stem

Figure 5.8. T.S of *Meyna laxiflora* stem bark

Figure 5.9. T.S of *Meyna laxiflora* root

Figure 5.10. T.S of *Meyna laxiflora* fruit
Pharmacognostic Evaluation of *Meyna laxiflora*

Figure 5.11. Powder microscopy of *Meyna laxiflora* leaf

Figure 5.12. Powder microscopy of *Meyna laxiflora* stem

Figure 5.13. Powder microscopy of *Meyna laxiflora* stem bark

Figure 5.14. Powder microscopy of *Meyna laxiflora* root
5.3.4. Physical parameter

The physical constant evaluation of the drugs play significant role in identification of adulteration or improper handling of drugs. Thus all parts of plant were subjected for physical evaluation. The results are mentioned in table 5.3.

The foreign organic matter was found to be 01.35 ± 0.02, 01.50 ± 0.01, 00.82 ± 0.02, 02.19 ± 0.05 and 01.67 ± 0.03 for leaf, stem, bark, root and fruit respectively which indicate that plant materials were collected with standard procedure and precautions taken during drying.

Loss on drying was found to be 09.51 ± 0.02, 07.82 ± 0.02, 07.69 ± 0.08, 11.65 ± 0.03 and 21.16 ± 0.09 % w/w for leaf, stem, bark, root and fruit respectively which indicate that plant materials have very low moisture content which will not able to produce microbial growth and deterioration of plant material.
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Total Ash value was found to be 13.58 ± 0.03, 11.80 ± 0.03, 10.63 ± 0.06, 09.60 ± 0.05 and 07.23 ± 0.06 % w/w for leaf, stem, bark, root and fruit respectively which indicates amount of inorganic radicals present in parts of plant like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. these compounds are available in definite amount in particular crude drug hence the data will help full for standardization of plant materials.

Water soluble ash was found to be 04.33 ± 0.03, 03.52 ± 0.03, 03.81 ± 0.04, 04.27 ± 0.05 and 02.56 ± 0.08 % w/w for leaf, stem, bark, root and fruit respectively which is determined for removing water soluble impurities from total ash.\(^7\)

Acid -insoluble ash was found to be 02.26 ± 0.03, 01.56 ± 0.01, 02.67 ± 0.06, 01.36 ± 0.02 and 01.31 ± 0.04 % w/w for leaf, stem, bark, root and fruit respectively which determined for removing inorganic variable from total ash like calcium oxalate, silica, carbonate and earthy matters.\(^7\)

Sulphated ash was found to be 12.35 ± 0.02, 14.24 ± 0.02, 11.79 ± 0.04, 07.99 ± 0.06 and 09.28 ± 0.04 % w/w for leaf, stem, bark, root and fruit respectively which also traces compound in small concentration.\(^10\)

The extractive value determined with different solvent with increasing polarity such as petroleum ether, chloroform, methanol and water for all parts of plant (leaf, stem, bark, root and fruit). The results shows all parts have considerable highest water soluble and alcohol soluble extractive value as compare other. Which indicates that plant material have significant concentration of poly phenols, flavonoids, saponins etc.\(^11\)
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Table 5.3. Quantitative microscopy of *Meyna laxiflora*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Values (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Bark</td>
<td>Root</td>
</tr>
<tr>
<td>1</td>
<td>Foreign organic matter</td>
<td>01.35 ± 0.02</td>
<td>01.50 ± 0.01</td>
<td>00.82 ± 0.02</td>
<td>02.19 ± 0.05</td>
<td>01.67 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>Loss on drying</td>
<td>09.51 ± 0.02</td>
<td>07.82 ± 0.02</td>
<td>07.69 ± 0.08</td>
<td>11.65 ± 0.03</td>
<td>21.16 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>Ash value</td>
<td>13.58 ± 0.03</td>
<td>11.80 ± 0.03</td>
<td>10.63 ± 0.06</td>
<td>09.60 ± 0.05</td>
<td>07.23 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>04.33 ± 0.03</td>
<td>03.52 ± 0.03</td>
<td>03.81 ± 0.04</td>
<td>04.27 ± 0.05</td>
<td>02.56 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>Acid insoluble ash</td>
<td>02.26 ± 0.03</td>
<td>01.56 ± 0.01</td>
<td>02.67 ± 0.06</td>
<td>01.36 ± 0.02</td>
<td>01.31 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>Sulphated ash</td>
<td>12.35 ± 0.02</td>
<td>14.24 ± 0.02</td>
<td>11.79 ± 0.04</td>
<td>07.99 ± 0.06</td>
<td>09.28 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>Pet ether soluble extractive value</td>
<td>02.14 ± 0.02</td>
<td>01.69 ± 0.03</td>
<td>00.78 ± 0.04</td>
<td>01.54 ± 0.02</td>
<td>01.28 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>Chloroform soluble extractive value</td>
<td>04.37 ± 0.03</td>
<td>03.00 ± 0.04</td>
<td>03.36 ± 0.06</td>
<td>05.27 ± 0.03</td>
<td>06.31 ± 0.04</td>
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<td>9</td>
<td>Alcohol soluble extractive value</td>
<td>06.42 ± 0.04</td>
<td>07.30 ± 0.05</td>
<td>04.55 ± 0.03</td>
<td>06.79 ± 0.01</td>
<td>04.32 ± 0.02</td>
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<tr>
<td>10</td>
<td>Water soluble extractive value</td>
<td>08.53 ± 0.03</td>
<td>06.43 ± 0.04</td>
<td>04.39 ± 0.04</td>
<td>06.39 ± 0.05</td>
<td>08.18 ± 0.05</td>
</tr>
</tbody>
</table>
5.4. References


