5. PHARMACOGNOSTICAL STUDIES

5.1 INTRODUCTION

Quality control of crude drugs and herbal formulation is of paramount importance in extenuating their acceptability in modern system of medicine, but the foremost problem encountered by the herbal drug industry is non-availability of standard quality control profile for herbal materials and their formulations (Verma & Singh 2008). Standardization is essential for ensuring the quality control of the herbal drugs. The misappropriation in herbal medicine or natural products begins with wrong identification. The most common error being one common vernacular name is given to two or more entirely different species (Dineshkumar 2007). It can be solved by pharmacognostic studies of medicinal plants. It is very important and in fact essential to lay down pharmacognostic specifications of medicinal plants which are used in various herbal drugs.

Pharmacognosy is the study of medicines derived from natural sources, mainly from plants, animal and mineral origin. This deals with standardization, authentication and study of natural drugs. Most of the research in pharmacognosy contributes to identifying controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, phytochemical and physicochemical analysis. The importance of pharmacognosy has been widely felt in recent times. Unlike taxonomic identification, pharmacognostic study includes various parameters, which help in identification of dried and powdered plant materials. Pharmacognostic study ensures plant identity, provide
standardization parameters which will be helpful in prevention of adulteration (Sumitra 2014).

5.2 MATERIALS AND METHODS

Collection of plant material

Fresh roots of Stereospermum colais (Buch. - Ham.ex Dillw.) Mabberley were collected from Alagarkovil hills, Madurai, Tamil Nadu and Stereospermum suaveolens were collected from Tirupati, Andhrapradesh. The plants were identified, confirmed (Gamble 1935, Henry et al 1987 & Mathew 1983) and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. The voucher specimen numbers of Stereospermum colais and Stereospermum suaveolens are PARC / 2007 / 80 & PARC / 2012 / 1080, respectively.

5.2.1 Anatomical Studies

Preparation of specimen

The root samples were cut and fixed in FAA [Farmalin (5 ml) + Acetic acid (5 ml) + 70% Ethyl alcohol (90 ml)]. After 24 h of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (Sass 1940). Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60° C) until TBA solution attained super saturation and 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 done by customary procedure (Johansen, 1940). The sections were stained with toluidine blue (O’Brien et al. 1964), since it is a polychromatic stain. Powdered materials were cleared with sodium hydroxide and mounted in glycerine medium. After staining, different cell components were studied using compound microscope.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphot 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, they appear bright against dark background under polarized light. Magnifications of the figures were indicated by the scale-bars. Descriptive terms of the anatomical features were as given in the standard anatomy books (Esau 1979).

5.2.2 DNA Sequencing Using ITS2 Markers

Authentication of plant specimens by DNA barcodes will be an effective, reliable and simple pharmacognostical tool to resolve the confusion in morphological identification (Selvarj et al. 2012). Due to different rates of evolution, nuclear ribosomal Internal Transcribed Spacer (ITS) regions have become the
routine marker in evolutionary studies at different taxonomic levels (Balasubramani et al. 2010 & Bertini L et al. 1999).

**Procedure**

**Extraction of DNA**

Genomic DNA was isolated from 100 mg of fresh leaf tissue (Saghai et al. 1984). Total cellular DNA was isolated from the fresh leaves by crushed with 15 ml of extraction buffer (50 mM Tris, pH 8.0, 0.7 M NaCl, 10 mM EDTA, 1 % hexadecyl trimethyl ammonium bromide, 0.1 % 2-mercapto ethanol) and incubated at 60°C for 30-60 min with occasional mixing by gentle swirling. To the above mixture, 10 ml of chloroform : octanol (24:1) was added and mixed by inversion to form an emulsion. Then it was centrifuged at 5000 rpm for 10 min at room temperature. The aqueous phase was removed and 2/3 volume of isopropanol was added and mixed by 2-4 quick, gentle inversions. The precipitated DNA was lifted out with a glass hook, transferred into 20 ml of solution containing 76 % ethanol and 10 mM NH₄OAc. After 20 min, 1.5 ml of solution containing 10 mM NH₄OAc and 0.25 mM EDTA was added. The DNA was isolated as dry tissue.

**DNA sequencing**

PCR amplification was done with 50 ng of total genomic DNA as template using ITS2: S2F (ATGCCGATATCTTGGTGTAAT), S2R (GACGCTTCTCCAGACTACAAT) (Chen et al. 2010). PCR amplification was done in a thermal cycler using initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 minute, final extension at
72°C for 5 min and held at 16°C. The PCR products were checked by agarose gel electrophoresis and purified using EZ-10 Spin Column PCR purification kit. The purified PCR products were sequenced in 3130xl Genetic analyzer.

5.2.3 Physico - Chemical Evaluation (Anonymous, 1996)

ASH VALUES

Total ash

About 5 gm of the root powder of Stereospermum colais and Stereospermum suaveolens were weighed and taken into a silica crucible which had been previously heated and cooled before weighing. The crucible was ignited by gradually increasing the temperature upto 450°C and was repeated until constant weight was obtained.

Water soluble ash

The ash was boiled with 25 ml of water and filtered through an ashless filter paper. The filter paper was rinsed with hot water and ignited in a silica crucible, cooled and then the ash present in the crucible was weighed. This represents the water insoluble ash. The water soluble ash was calculated by subtracting the water insoluble ash from the total ash.

Acid insoluble ash

The ash was boiled with 25 ml of diluted Hydrochloric acid for 5 min and filtered through an ash less filter paper. Then the filter paper was ignited in a silica crucible, cooled and then weighed.
Sulphatedash

About 5 gm of the powdered root material was taken in a crucible and ignited to char. Then the residue was moistened with 1 ml of concentrated sulphuric acid and once again ignited. The ash obtained was cooled and weighed.

Ethanol soluble extractive

About 5 gm of dried coarse powder of roots of *Stereospermum colais* and *Stereospermum suaveolens* were macerated with 100 ml of 90 % ethanol in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. Then it was filtered immediately; 25 ml of the filtrate was evaporated to dryness in a tarred china dish at 105°C and then weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried drug.

Water soluble extractive

About 5 gm of coarse powder of roots of *Stereospermum colais* and *Stereospermum suaveolens* were taken and dissolved in 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.

Crude fibre content

About 2 gm of the powdered root of *Stereospermum colais* and *Stereospermum suaveolens* were defatted, boiled with sulphuric acid for 30 min followed by sodium hydroxide, and finally with water and alcohol. The residue was dried and incinerated. The percentage crude fibre content was calculated.
Loss on drying

A quantity of 1 gm of powdered root of *Stereospermum cola* and *Stereospermum suaveolens* were taken in a glass stoppered weighing bottle and dried in a 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.

5.2.4 Elemental Analysis

The mineral contents of medicinal plant species play a key role in the proper functioning of the vital organs as well as in the promotion of the general well-being of the body. However, they may be toxic if consumed beyond their estimated safe daily intake. Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants is obvious. Since plants and animals are essential sources of micronutrients for man, it becomes necessary to monitor the levels in biological materials that are required by man for both dietary and medicinal purposes (Jarup 2003). Heavy metals have the tendency to accumulate in both plants and human organs. The micronutrients can be good, toxic or lethal depending on the dose. Thus elemental analysis is essential to ascertain the safety of the plant material (Llobet et al. 2003).

Procedure

The powdered root material of *Stereospermum cola* and *Stereospermum suaveolens* were taken and the minerals and heavy metals content was analysed using Atomic Absorption Spectroscopy.
5.3 RESULTS AND DISCUSSION

5.3.1 Macroscopical Characters

The young roots of *Stereospermum colais* and *Stereospermum suaveolens* were taken and the organoleptic and morphological features were observed and the observations were presented in Table 5.1.

**Table 5.1 Macroscopical characters of the roots**

<table>
<thead>
<tr>
<th>Description</th>
<th>Stereospermum colais</th>
<th>Stereospermum suaveolens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellowish brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter and astringent</td>
<td>Astringent</td>
</tr>
<tr>
<td>Shape</td>
<td>Cylindrical</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>Fracture</td>
<td>Fibrous</td>
<td>Short</td>
</tr>
<tr>
<td>Surface</td>
<td>Fissures with lenticels are observed</td>
<td>Fissures are deep with transversely extended lenticels</td>
</tr>
</tbody>
</table>

![Figure 5.1 Surface morphology of the roots](image)

(a) *Stereospermum colais* (lenticels)  
(b) *Stereospermum suaveolens* (fissures)
5.3.2 Anatomical Studies

5.3.2.1 Transverse section of root of *Stereospermum colais*

It consists of thin periderm, wide cortex, secondary phloem and central solid cylinder of secondary xylem [Figure 5.3 (1, 2)]. The periderm is superficial and it varies in thickness from 3 to 5 layers of phellem cells which are tubular in shape and suberized [Figure 5.3 (1, 2)]. A single layer of phellem cells in the middle of the periderm becomes thick walled and lignified so that the phellem is heterogeneous in nature. This lignified layer of phellem cells is called phelloid layer. Inner to the periderm is a wide cortex, which includes parenchymatous cells with thin walls.

Secondary phloem occurs inner to the cortex but the border between cortex and secondary phloem is not evident. The secondary phloem zone consists of dilated rays and thin discontinuous segments of fibre occur in circular cylinder in the phloem. The fibre cylinders are broken by phloem rays. The sieve elements are very small, angular in outline and have small companion cells at one corner of the cells (Figure 5.5). The secondary xylem cylinder is 900 µm in diameter. It includes diffusely distributed wide circular thin walled vessels and xylem fibres. The vessels
may be solitary or in multiples of two. There are upto 70 µm in diameter. The xylem fibres have thin walls and wide lumen. The xylem rays are in thin straight radial lines (Figure 5.4).

The phloem rays traverse the sclerenchyma cylinders. The sieve elements are intact and they are angular in shape with distinct companion cells [Figure 5.5 (1)]. The xylem vessels are mostly in multiples of two, measuring 40 µm in diameter. Xylem fibres are thick walled with wide lumen [Figure 5.5 (2)].

5.3.2.2 Transverse section of the root bark of *Stereospermum colais*

The bark is rough surfaced with minute fissures. It consists of a thin superficial periderm and wide cortex followed by secondary phloem [Figure 5.6 (1)]. The cortical cells are tangentially elongated and compressed. They are large cells and compactly arranged [Figure 5.6 (2)]. The secondary phloem is differentiated into outer wide zone of collapsed phloem and inner narrow zone of non-collapsed phloem. The collapsed phloem is wider and includes several scattered masses of fibres and dilated rays [Figure 5.6 (2) & 5.7 (1)]. There is a distinct boundary between collapsed and non-collapsed phloem [Figure 5.7 (1)]. Fibres are absent in the non-collapsed phloem. The sieve elements are polygonal in outline, thick walled and have distinct companion cells [Figure 5.7 (2)]. Calcium oxalate druses are occasionally seen in the collapsed phloem parenchyma cells [Figure 5.7 (3)].

5.3.2.3 Tangential longitudinal section of the phloem of *Stereospermum colais*

In T.L.S view, the rays appear non-storied and they are uniseriate, in part multiserrate or fully multiseriate. The rays have long upright cells and squarish procumbent cells [Figure 5.8 (1, 2)]. The rays are 400 µm in height and 30 µm in breadth. Some of the uniseriate rays are 200 µm in height. The phloem parenchyma
cells occur in vertical strand. The cells are rectangular and vertically elongated. The phloem fibres are long and narrow, thick walled with wide lumen [Figure 5.8 (3)].

5.3.2.4 Powder Microscopy of root of *Stereospermum colais*

The powder preparation of the root material exhibits the following inclusions

(i) Fibres:

The fibres are long, narrow, thick walled with tapering ends. The fibre walls are very thick and the cell lumen is very narrow [Figure 5.9 (1, 2 & 3)]. The fibre wall is 20 µm thick.

(ii) Periderm:

There are small fragments of periderm tissues seen in surface view. The cells are radially elongated, compact and parallel to each other. The cell walls are suberized and thick walled. The periderm is homocellular.

(iii) Lipid bodies:

There are free floating, spherical, dark red lipid bodies of different sizes are seen floating in the powder. They are stained with neutral red and turns to red colour on staining(Figure 5.10).

5.3.2.5 Transverse section of root and root bark of *Stereospermum suaveolens*

The root bark exhibits highly complex structure and organization. The periderm is multiple types, which is known as rhytidome. The first formed periderm is quite thick and highly undulate having deep furrows and thick ridges [Figure 5.11(1)]. The periderm also has deep, wide and irregular fissures. The periderm is 400 µm thick. The periderm includes outer wider phellem and inner narrow
phelloderm. The phellem cells are tabular in shape, suberised and the cells occur in regular vertical rows. The phelloderm cells are also tabular in shape but, they are darkly stained and the cell walls have cellulose content [Figure 5.11 (1)].

When the first periderm stops its activity, a second periderm or sequent periderm originates in the deeper region of the cortex or secondary phloem. The second sequent periderm is formed in the form of deep loop and the two ends of the loop are fused with the first periderm. The cortical tissue or secondary phloem is enclosed within the loop of the sequent periderm [Figure 5.12 (1)].

The second periderm is also thick, with the cells being tabular in shape and suberised. The inner bark is the secondary phloem, which is thicker than the outer bark (Rhytidome). The secondary phloem is differentiated into outer wider zone of collapsed phloem and inner zone of narrow non-collapsed phloem. The collapsed phloem consists of highly dilated phloem rays and phloem parenchyma cells. Phloem fibres are diffusely distributed in the collapsed phloem [Figure 5.11 (2)]. The sieve elements are crushed into dark masses or lines.

5.3.2.6 Tangential longitudinal section of the phloem of Stereospermum suaveolens

The phloem rays appear non storied; i.e., the height of the rays are at various levels. The rays are at various levels. The rays are uniseriate, biseriate and multiseriate. Multiseriate rays are more common (Figure 5.13). The rays are thin and very high. The uniseriate rays are upto 280 µm in height. The multiseriate rays are upto 320 µm height and 50 µm thick. The rays are heterocellular; the rays consist of middle portion of squarish or horizontally elongated procumbent cells and at the end of rays.
5.3.2.7 Crystal distribution in the root of *Stereospermum suaveolens*

Calcium oxalate crystals are fairly abundant in the cortical portion of the root. There are two types of crystals in the cortical cells. In the outer portion of the cortex, the crystals are raphide type. The raphide consists of thin, long pointed needles compactly bundled into spindle shaped bodies (Figure 5.14). In the inner part of the cortex, the crystals are druses. They are spherical bodies which are formed by the aggregation of compactly arranged, small pointed prismatic crystals (Figure 5.14).

5.3.2.8 Secondary xylem in the root of *Stereospermum suaveolens*

Secondary xylem occurs in thick, circular, compact cylinder [Figure 5.12 (2)]. The secondary xylem exhibits 1 or 2 growth rings in which the vessels are ring porous. In the beginning of the growth ring the vessels are wider, circular and thin walled. Towards the end of the growth the vessels are narrow, less in frequency and thick walled. The vessels are mostly solitary, rarely in multiples of two. They have thick walls, the early wood vessels are 50 µm wide and the late wood vessels are 20 µm wide. The xylem fibres are thick walled, lignified and arranged in compact radial rows (Figure 5.15).

5.3.2.9 Powder Microscopy of root of *Stereospermum suaveolens*

In the powder, small fragments of periderm cells are seen in surface view. The cells are phellem and they have thick radial walls [Figure 5.16 (1, 2)]. The cells are radially oblong and compact. Spherical darkly stained red lipid bodies are very frequent in the powder. When stained with neutral red or sudan dyes these bodies turn red indicating that they are lipids [Figure 5.16 (3)].
Table 5.2 Salient anatomical features of *Stereospermum colais* and *Stereospermum suaveolens*

The following characters were used to differentiate the two plants

<table>
<thead>
<tr>
<th>S.No</th>
<th>Stereospermum colais</th>
<th>Stereospermum suaveolens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Periderm (Hetero cellular)</td>
<td>Periderm (Multiple type - Rhytidome)</td>
</tr>
<tr>
<td></td>
<td>a. Suberized phellem cells</td>
<td>1. First periderm – thick and undulate</td>
</tr>
<tr>
<td></td>
<td>b. Lignified phellem cells (Phelloid layer)</td>
<td>a. Outer wide Phellem (suberized)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Inner narrow phelloderm (Cellulose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 2(^{nd}) periderm or sequent periderm which forms a deep loop enclosed of cortex and secondary phloem.</td>
</tr>
<tr>
<td>2.</td>
<td>Secondary phloem: Dilated rays are infrequent</td>
<td>Secondary phloem: Dilated rays are widespread</td>
</tr>
<tr>
<td>3.</td>
<td>Sieve elements are in regular radial files</td>
<td>Sieve elements are irregular in arrangement comprising small polygonal elements</td>
</tr>
<tr>
<td>4.</td>
<td>Phloem rays are mostly uniseriate, occasionally biseriate and multiseriate</td>
<td>Phloem rays are uniseriate, less frequently multiseriate</td>
</tr>
<tr>
<td>5.</td>
<td>Phloem fibres occur in small discrete clusters in tangential bands</td>
<td>Phloem fibres are distorted and scattered due to dialation of the phloem rays</td>
</tr>
<tr>
<td>6.</td>
<td>Vessels uniformly distributed from center to periphery, both solitary but frequently radial multiples of 2 or 3 and about 70 (\mu m) in diameter</td>
<td>Central cylinder with small, narrow vessels in outer zone with wider inner vessels, distribution is diffuse and scattered. Vessels are solitary about 20-50 (\mu m) in diameter</td>
</tr>
<tr>
<td>7.</td>
<td>Calcium oxalate druses are seen in the collapsed phloem parenchyma cells</td>
<td>Calcium oxalate crystals are seen as raphides in the inner cortex and as druses in the outer cortex</td>
</tr>
</tbody>
</table>
5.3.3 DNA Sequencing using ITS2 Markers

Recent molecular methods like DNA barcoding have been extensively used for species identification, diversity, forensic medicine and ecological studies (Pereira et al. 2008 & Ferri et al. 2012). It also plays an important role in identification of traditional medicinal herbs. ITS2 has been extensively used in differentiating morphologically similar species (Gao et al. 2010).

5.3.3.1 Stereospermum colais - Full length 471 base pair

GCAGAATCCCGTGAAACCATCGAGTCTTTTGAAACGCAAGTTGCGCCC
GAAGCCATTAGGCTAGGGACGACGTCTGCTTTGGCGCTCCTGCATCGGCTC
GCCCCCCTCCACTCGCCTGCCGAGGGTTTGGTCTGAGGCGGATGTTTG
GCCCCCGTGCGCTCTCTGGCGCGCCGGCCGCCCCAAATCGGAACCCCGGCG
GATGCACTAGTCAGTGAGGTGGTGAGGAACTCAACTCTTTTGCTGGCG
TGCTAGACGTGCTGCTCTGCGGGGAAACATCAATGACCCATAGGCGCCTT
GCTCGCGTGTCTCGGCTCGCAGCCGCGTCTCGGACCCCGGACCCCAG
GTCAGGCGGGAATTACCCCGCTGAGTTTAAAGCATATCAATAAAGCGGAGGAA
AAGAAACTTACAAGGATTTCCCTAGTAAACCGCGAGCGAACCAGGGAATAG
CCCAACTTGAGAATCCGGCGTCGCGCTCGCCGCTCCG

5.3.3.2 Stereospermum suaveolens - Full length 471 base pair

GCAGAATCCCGTGAAACCATCGAGTCTTTTGAAACGCAAGTTGCGCCC
GAAGCCATTAGGCTAGGGACGACGTCTGCTTTGGCGCTCCTGCATCGGCTC
GCCCCCCTCCACTCGCCTGCCGAGGGTTTGGTCTGAGGCGGATGTTTG
GCCCCCGTGCGCTCTCTGGCGCGCCGGCCGCCCCAAATCGGAACCCCGGCG
GATGCACTAGTCAGTGAGGTGGTGAGGAACTCAACTCTTTTGCTGGCG
TGCTAGACGTGCTGCTCTGCGGGGAAACATCAATGACCCATAGGCGCCTT
GCTCGCGTGTCTCGGCTCGCAGCCGCGTCTCGGACCCCGGACCCCAG
GTCAGGCGGGAATTACCCCGCTGAGTTTAAAGCATATCAATAAAGCGGAGGAA
AAGAAACTTACAAGGATTTCCCTAGTAAACCGCGAGCGAACCAGGGAATAG
CCCAACTTGAGAATCCGGCGTCGCGCTCGCCGCTCCG
In this study the DNA sequence using ITS2 markers were obtained and compared. The genomic DNA was isolated from *Stereospermum colais* and *Stereospermum suaveolens* [Figure 5.17 (a)] and used for PCR amplification using ITS2 markers. The PCR-amplified ITS2 regional sequences showed similar nucleotide sequences in both the plants [Figure 5.17 (b)].

(a) Genomic DNA  
(b) ITS2- PCR product of *Stereospermum colais* and *Stereospermum suaveolens*  

(IPTS - Internal Transcribed Sequence 2 marker, SC - *Stereospermum colais*, SS - *Stereospermum suaveolens*)

Figure 5.17 Electrophoretic profiles of SC and SS using ITS2 markers
5.3.4 Physico-Chemical Constants

Table 5.3 Physico-chemical constants

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Percentage w/w</th>
<th>Stereospermum colais</th>
<th>Stereospermum suaveolens</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>ASH VALUES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>2.54 ± 0.12</td>
<td>6.83 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>0.12 ± 0.04</td>
<td>2.91 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Water soluble ash</td>
<td>1.13 ± 0.43</td>
<td>2.25 ± 0.62</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Sulphated ash</td>
<td>0.86 ± 0.27</td>
<td>1.12 ± 0.78</td>
<td></td>
</tr>
<tr>
<td>II.</td>
<td>EXTRACTIVE VALUES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Ethanol Soluble Extractive</td>
<td>4.81 ± 0.18</td>
<td>2.17 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Water Soluble extractive</td>
<td>5.63 ± 0.32</td>
<td>4.15 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>CRUDE FIBRE CONTENT</td>
<td>1.27 ± 0.24</td>
<td>1.69 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>LOSS ON DRYING</td>
<td>8.10 ± 2.43</td>
<td>7.98 ± 1.98</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD of triplicates

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physico-chemical constants such as total ash, acid insoluble ash, water soluble ash, water and alcohol extractive values, crude fibre content and loss on drying were determined (as per the procedure of Indian Pharmacopoeia). The percentage content was estimated and tabulated (Table 5.3).
5.3.5 Elemental Analysis

The amount of heavy metals such as lead, cadmium, mercury and arsenic was estimated in the roots of Stereospermum colais and Stereospermum suaveolens. It was found that the levels were within the limit of WHO standards (WHO 2006) in both the plants. Also the amount of other elements such as copper, zinc, sodium, potassium, selenium and iron was estimated and the results were tabulated (Table 5.4).

Arsenic is a toxic non-essential element, which acts by affecting about 200 enzymes particularly those involved in DNA synthesis and repair. Acute arsenic poisoning is associated initially with nausea, vomiting, abdominal pain, severe diarrhea, encephalopathy and peripheral neuropathy. Chronic toxicity results in multisystem diseases including carcinogenesis and affecting almost all organs (Ratnaike 2003). The amount of arsenic present in the roots was less than the limit i.e. 5 ppm. as per WHO guidelines.

Lead can complex with various biomolecules and adversely affect their functions. Lead exposure may have an adverse effect on the blood, nervous, immune, renal, skeletal, muscular, reproductive and cardiovascular systems causing poor muscle coordination, gastrointestinal symptoms, brain and kidney damage, hearing and vision impairments, and reproductive defects (Johnson 1998). The amount of lead present in the roots was less than the limit i.e. 10 ppm. as per WHO guidelines.
Table 5.4 Elemental analysis of roots of *Stereospermum colais* & *Stereospermum suaveolens*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the element</th>
<th><em>Stereospermum colais</em></th>
<th><em>Stereospermum suaveolens</em></th>
<th>WHO standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lead</td>
<td>3.12 ppm</td>
<td>5.45 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>2.</td>
<td>Cadmium</td>
<td>0.19 ppm</td>
<td>0.24 ppm</td>
<td>1 ppm</td>
</tr>
<tr>
<td>3.</td>
<td>Mercury</td>
<td>Nil</td>
<td>0.012 ppm</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>4.</td>
<td>Arsenic</td>
<td>0.12 ppm</td>
<td>0.45 ppm</td>
<td>5 ppm</td>
</tr>
<tr>
<td></td>
<td>Percentage w/w</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Copper</td>
<td>2.31 mg</td>
<td>1.22 mg</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Zinc</td>
<td>3.8926 mg</td>
<td>4.364 mg</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Sodium</td>
<td>63.6 mg</td>
<td>57.6 mg</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Potassium</td>
<td>89.4 mg</td>
<td>120.6 mg</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Selenium</td>
<td>2.35 µg</td>
<td>1.35 µg</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Iron</td>
<td>4.108 mg</td>
<td>13.47 mg</td>
<td>-</td>
</tr>
</tbody>
</table>

High levels of cadmium possess a serious toxicological effect on human health. Kidney is the critical target organ in the exposed population. Excretion of cadmium is very slow and it accumulates in human kidney for a relatively longer time, resulting in an irreversible impairment of the renal tract (Li et al. 2012). Cadmium level in both the plants were within the permissible limit i.e. 1 ppm. as per WHO guidelines.
Mercury can cause adverse effects on the renal and nervous systems and can cross the placental barrier and produce potential toxic effects on the fetus. Levels of mercury beyond the allowable limits have been associated with infertility, inhibition of endogenous antioxidant enzymes and brain damage (Anonymous 2003). Mercury was found to be nil in *Stereospermum colais* and found to be less than the limit i.e. 0.5 ppm as per WHO guidelines in *Stereospermum suaveolens*.

The maximum permissible level (MPL) of iron is 1000 µg/day. Iron salts have an astringent action resulting in irritation of the gastrointestinal mucosa, which gives rise to gastric discomfort, nausea, vomiting and diarrhea or constipation (Obi et al. 2006 & Bourman & Rand 1980). The amount of iron present in both the roots was within the permissible level per 10 gm daily dose of plant material.

Zinc is an element of many metalloenzymes, particularly certain enzymes of nucleic acid metabolism (Atukorala & Waidyanatha 1987). Also act as a membrane stabilizer and stimulator of the immune response (Das & Dasgupta 2002). Acute zinc poisoning is characterized by nausea, vomiting, diarrhoea, fever and lethargy. The estimated safe and adequate daily intake of zinc is between 10,000 and 20,000 µg/day (Obi et al. 2006). The zinc levels per 10 gm of plant species were lower than the maximum permissible level and regarded as safe.

Copper is a component of many enzyme systems, such as cytochrome oxidase, lysyl oxidase and ceruloplasmin. However, copper could be toxic depending on the dose and duration of exposure (Rania et al. 2015). The Maximum Permissible Level (MPL) of copper is 12,000 µg/day (Obi et al. 2006). Therefore, the suggested average intake of about 10 g of plant material was lower than the permissible level.
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, haemolysis etc. The maximum permissible level (MPL) of selenium is 400 µg/day. Therefore, the suggested average intake of about 10 g of plant material was lower than the permissible level.

Sodium and potassium take part in ionic balance of the human body and maintenance of tissue excitability, carry normal muscle contraction and help in formation of gastric juice in stomach (Brody 1998). Sodium level is found to be less than potassium in both the roots. Potassium is important as diuretic and it takes part in ionic balance of the human body and maintains tissue excitability. Potassium is the chief intracellular cation and plays a vital role in the transmission of electrical impulse in the nerve cells. (Venkataraman & Gopalakrishnan 2002). The potassium concentration was found to be more in Stereospermum suaveolens (120.6 mg %) than Stereospermum colais (89.4 mg%) which may attribute to the diuretic effect of Stereospermum suaveolens reported by Balasubramanian et al. (2009).

5.4 CONCLUSION

The pharmacognostical parameters such as macroscopy, microscopy, physico-chemical analysis and elemental analysis were carried out to standardize the roots of Stereospermum colais and Stereospermum suaveolens. The root of Stereospermum colais is yellowish brown in colour, bitter and astringent in taste whereas, Stereospermum suaveolens is dark brown in colour and astringent in taste. The fissures observed in the outer surface are more and deep in Stereospermum suaveolens than in Stereospermum colais. In the microscopical studies, heterocellular periderm is observed in Stereospermum colais and multitype (Rhytidome) periderm is observed in Stereospermum suaveolens. Calcium oxalate druses are observed in the
collapsed phloem region of *Stereospermum colais* while in *Stereospermum suaveolens*, the crystals are exist as raphides in inner cortex and as druses in the outer cortex region. DNA fingerprint of the plants were taken using ITS2 markers, the PCR-amplified ITS2 regional sequences showed similar nucleotide sequences in both the plants.

Physico-chemical analysis showed that the ash value was found to be more in *Stereospermum suaveolens* (6.83 %) than in *Stereospermum colais* (2.54 %). The extractive values of *Stereospermum colais* such as ethanol soluble (4.81 %) and water soluble extractive (5.63 %) values were found to be more than *Stereospermum suaveolens* ie 2.17 % and 4.15 % respectively. The elemental analysis of the roots revealed that the levels of heavy metals such as lead, cadmium, mercury and arsenic were found to be within the limit as per WHO standard in both the plants. When analyzing the other elemental composition of the plants, copper, sodium and selenium content was found to be more in *Stereospermum colais* while *Stereospermum suaveolens* was found to be rich in zinc, potassium and iron content.

The results obtained from this study will certainly help in identification and authentication of the plant material. The standardization parameters that have been studied here can be used as reference standard for correct identification of the plant in future and also will be useful in making a monograph of the plant. Further, it acts as a tool to detect adulterants and substituents accordingly maintains the quality, reproducibility and efficacy of the plant material.

5.5 REFERENCES


• Balasubramanian, T, Karthick, P & Tapan Kumar C 2009, ‘Diurectic effect of ethanol extract of *Stereospermum suaveolens*’, *Pharmacology online*, vol. 2, pp. 625-635.


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