12. SUMMARY

The plants selected for this study, *Stereospermum colais* and *Stereospermum suaveolens* are named as “Patala” in Ayurvedic medicine. The roots of “Patala” have rich traditional value especially in the treatment of inflammation and rheumatism also one of the ingredients in Dasamoola. Even though “Patala” is a reputed Ayurvedic plant used for various ailments, there is a lacunae in the authentication and identification of the root of the two plants and the best species having anti-arthritic potential. Hence, the aim of the study was to compare the pharmacognostical, phytochemical and pharmacological studies of the roots of *Stereospermum colais* and *Stereospermum suaveolens*.

Pharmacognostical studies

- Roots of *Stereospermum colais* and *Stereospermum suaveolens* were collected and authenticated.

- 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 and bitter in nature, whereas *Stereospermum suaveolens* is dark brown in colour, the fissures observed in the outer surface are more and deep than *Stereospermum colais*.

- Through the microscopical studies, heterocellular periderm is observed in *Stereospermum colais* and multi-type (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 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 ethanol soluble (4.81 %) and water soluble extractive (5.63 %) values of *Stereospermum colais* were found to be more than *Stereospermum suaveolens* (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 compositions of the plants such as, copper, sodium and selenium content, they were found to be more in *Stereospermum colais* while *Stereospermum suaveolens* was found to be rich in zinc, potassium and iron content.

**Phytochemical studies**

- Successive solvent extracts of both the plants were prepared. The yield of the extracts was found to be more in *Stereospermum colais* than that of *Stereospermum suaveolens*. 
• Preliminary phytochemical analysis of powdered root materials and various extracts were carried out which provides information regarding the presence of specific phytoconstituents in each species. *Stereospermum colais and Stereospermum suaveolens* showed the presence of steroids, flavonoids, quinones, phenols, tannins, glycosides, carbohydrates, saponins, proteins and aminoacids. Both the plants differ in the presence of terpenoids and anthraquinones which were present in *Stereospermum suaveolens* and absent in *Stereospermum colais*.

• The fluorescence character of powdered root material and various extracts of *Stereospermum colais* and *Stereospermum suaveolens* were entirely different from each other.

• Quantification of primary and secondary metabolites were carried out which can be used as a standardization/authentification tool in future. The tannin and flavonoid content was found to be more in *Stereospermum suaveolens* (tannins – 36 % and flavonoids – 49.82 %) than *Stereospermum colais* (tannins – 26.66 % and flavonoids – 45.06 %).

• HPTLC fingerprinting of various extracts of roots of both species were taken, which helps in authentication of the specific species on comparison of the fingerprints.

• HPTLC profile of petroleum ether extracts showed 14 and 10 polyvalent peaks in PESC and PESS respectively, in which one common peak is observed at the $R_f$ value of 0.29.

• HPTLC profile of chloroform extracts showed 7 and 10 polyvalent peaks in CESC and CESS respectively, in which two similar peaks were observed at the $R_f$ value of 0.15 and 0.74.
HPTLC profile of ethyl acetate extracts showed 15 and 13 polyvalent peaks in EASC and EASS respectively, in which three common peaks were observed at the R<sub>f</sub> value of 0.04, 0.52 and 0.97.

HPTLC profile of ethanol extracts showed 13 and 15 polyvalent peaks in EESC and EESS respectively, in which two common peaks were observed at the R<sub>f</sub> value of 0.17 and 0.57.

Among the HPTLC profiles of various extracts of *Stereospermum colais* and *Stereospermum suaveolens*, the chloroform extract exhibited less number of peaks compared with other extracts which indicates that less number of chloroform soluble constituents.

EESC and EESS were standardized using lapachol by HPLC method and the amount of lapachol was found to be 19.5 % and 4.7 % respectively. The study revealed that *Stereospermum colais* contains high quantity of lapachol than *Stereospermum suaveolens* which may attribute for the greater efficacy of *Stereospermum colais* than *Stereospermum suaveolens*.

**Pharmacological studies**

*In vitro* antioxidant studies of various extracts of both plants were performed using DPPH radical and Nitric oxide radical scavenging assay. The results revealed that EESC and EESS showed a significant effect than other extracts and *Stereospermum suaveolens* (91.17 % and 70.84 %) was found to be more effective than *Stereospermum colais* (78.65 % and 58.42 %).
In vitro anti-arthritic activity of various extracts was performed by protein denaturation method. Among the various extracts of *Stereospermum colais* and *Stereospermum suaveolens*, the ethanolic extracts EESC (59.89 %) and EESS (49.40 %) showed maximum activity. Thus, the anti-arthritic activity of *Stereospermum colais* was found to be more than *Stereospermum suaveolens*.

HRBC membrane stabilization assay was carried out using various extracts of both species to screen the anti-inflammatory potential and the results revealed that EESC and EESS possessed a significant effect than other extracts and thus *Stereospermum colais* (61.72 %) was found to be potent than *Stereospermum suaveolens* (52.76 %).

In vitro anti-inflammatory activity of EESC and EESS were screened by TPA induced inflammation in polymorphonuclear leukocytes. Both the extracts showed efficacy against TNF-α, Cathepsin D levels and nitric oxide synthase activity. The EESS showed potency against the Cathepsin D level and nitric oxide synthase activity than EESC. EESC showed maximum activity in the reduction of TNF-α level. It is concluded that *Stereospermum colais* is potent than *Stereospermum suaveolens*.

In vivo acute toxicity study was carried out using EESC and EESS. The extracts were found to be safe and the LD$_{50}$ was greater than 2000 mg /kg b.wt. Hence, the extracts “EESC and EESS” falls in the “category-5 or unclassified” in accordance to the Globally Harmonised System of classification of chemicals.

In vivo antiarthritic activity of EESC and EESS were evaluated using Complete Freund’s Adjuvant induced arthritic model. EESC (58.97 %) and EESS (20.51 %) at the dose of
400 mg/kg b.wt. significantly reduced the paw volume which indicates the anti-inflammatory potential of the plants.

- The levels of biochemical markers such as LDH, ALP, AST, ALT, total protein, urea, creatinine, and C-reactive protein were studied. Both the extracts at the dose of 400 mg/kg b.wt. significantly reduced the elevated level of the above mentioned biochemical markers. Thus the extracts play an imperative role by reducing the prognosis of disease pathology.

- Histopathological studies on paw tissue and ankle joint revealed that EESC at the dose of 400 mg/kg b.wt. significantly reduced the synovitis, panniculitis, muscular necrosis and inflammatory cells infiltration whereas moderate reduction was observed in EESS at the dose of 400 mg/kg b.wt. treated group.

- Immunohistochemical studies on inflammatory markers revealed EESC at the dose of 400 mg/kg b.wt. significantly reduced the activation and proliferation of CD4 cells and the expression of IL-2 and TNF-α while EESS at the dose of 400 mg/kg b.wt. showed moderate reduction in the level of above markers. EESC & EESS treated groups at the dose of 200 mg/kg significantly reduced the TGF – β expression, whereas moderate reduction was observed in standard as well as the extracts treated group at the dose of 400 mg/kg. From the findings, the study implies that the extracts having immuno-modulatory effect similar to that of methotrexate, a disease modifying agent.

- The study revealed that EESC and EESS showed significant anti-arthritis effect. The presence of phytochemicals such as terpenoids, flavonoids, quinones, phenols and tannins
may probably attribute to the anti-arthritic property of the plants. *Stereospermum colais*

was found to be more potent than *Stereospermum suaveolens* which may be due to the

high content of lapachol.