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Identification of *Patala* (*Stereospermum colais* and *Stereospermum suaveolens* roots) by pharmacognostic parameters - A plant drug in Dasamula

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Stereospermum colais and *Stereospermum suaveolens* are known as "Patala" in Ayurveda and also a constituent of Dasamula. It is difficult to distinguish between the two species and hence it is difficult to identify genuine Patala. Both *Stereospermum* species looks alike in morphology with the exception of flower-color. So collection of the plant material from wild source as well as differentiating from the marketed sample is a challenge for the herbal industries to identify the genuine Patala. Hence, an attempt was made to compare the pharmacognostical and phytochemical parameters of the roots of *S. colais* and *S. suaveolens.* Macroscopy, microscopy, physico-chemical analysis and elemental analysis were carried out to standardize the roots. The salient diagnostic features identified to distinguish the plant species are heterocellular periderm and calcium oxalate druses in *S. colais* and multitype (Rhytidome) periderm and calcium oxalate raphides in *S. suaveolens*. Qualitative and quantitative phytochemical analysis and comparative HPTLC fingerprint analysis of various extracts of roots revealed their phytochemical composition. The standardization parameters developed here can be used as reference standard for correct identification of the plant. Further, it will act as a tool to detect adulterants and substituents consequently maintains the quality, reproducibility and efficacy of the plant material.

Keywords: HPTLC, Patala, Pharmacognosy, Physico-chemical, Phytochemical, *Stereospermum*

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Quality control of crude drugs and herbal formulation is of supreme importance in extenuating their suitability in modern system of medicine, but the leading problem encountered by the herbal drug industry is inadequacy of standard quality control profile for herbal materials and their formulations[1]. The misappropriation in herbal medicine arises with erroneous identification. The widespread error is being common vernacular name for various entirely dissimilar species[2]. It can be resolved by pharmacognostic studies to ensure plant identity, provide standardization parameters consequently prevent adulteration[3].

Stereospermum colais and *Stereospermum suaveolens* are known as "Patala" in Ayurveda and also a constituent of Dasamula. The roots are reported as diuretic, cardiotonic, anti-inflammatory, antibacterial, and febrifuge[4,5]. Both the roots are reported to contain β-sitosterol, lapachol and sterequinone[6]. The roots and stem bark were affirmed to possess antidiabetic, anticancer and anti-inflammatory activity[7-10]. The authors reported the phytochemistry, antioxidant and anti-arthritic potential[11,12]. *Saligrama nighantu* acknowledge three varieties of *patala viz., bhumipatala, ksudrapatala and vallipatala.* Conversely, Bhavaprakasa has stated two varieties namely, patala (white flowered) and the tamrapatala (copper-red flowered). Chunekar (1982)[13], in his annotations on chyavanprakasa has associated the patala with *S. colais* and tamrapatala with *S. suaveolens*. Most authors nevertheless do not make distinction of these types and accept *S. suaveolens* as the plant source[14-16]. Kerala physicians, however depend on *S. colais* for the plant source. Rheede[17] also portrays *S. colais* as the source of 'Padri'(in Tamil).

Even though the monograph of *S. suaveolens* is part of Ayurvedic Pharmacopoeia of India[18], there is lack of information about *S. colais*. Both the plants are

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alike in morphology with the exception of flower color. Hence, collection of the plant material from wild source as well as identification of the marketed sample is a massive challenge. Hence, the authors believed that a systematic study on these plants will help in identification of the plant besides providing standardization parameters for the quality control of the plants.

Materials and Methods

Plant material and processing

Fresh roots of *Stereospermum colais* (Buch. - Ham.ex Dillw.) Mabberley were collected from Alagarkovil hills, Madurai, Tamil Nadu and *Stereospermum suaveolens* (Roxb.) DC were collected from Tirupati, Andhra Pradesh in the month of November and authenticated by Prof P Jayaraman, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. The voucher specimen numbers of *S. colais* and *S. suaveolens* are PARC / 2007 / 80 & PARC / 2012 / 1080, respectively. They were deposited in the Pharmacognosy department museum. A portion of the freshly collected root material was washed thoroughly, cut into small pieces and immediately fixed in FAA solution for microscopical studies and the remaining were shade dried.

Macroscopical evaluation

The young roots of *S. colais* and *S. suaveolens* were taken and the organoleptic and morphological features were observed.

Microscopical evaluation

The root samples were cut into small pieces of 2 mm size and fixed in FAA [Formalin (5 mL) + Acetic acid (5 mL) + 70% Ethyl alcohol (90 mL)]. After 24 h of fixing, dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60°C) and the specimens were cast into paraffin blocks. The sections with 10-12 µm thickness were prepared with the help of rotary microtome. Dewaxing of the sections were done by customary procedure[19] and stained with toluidine blue[20]. Photographs were taken with Nikon labphot 2 microscopic unit. For the study of crystals, polarized light was employed and the magnifications of the figures were indicated by the scale-bars.

Powder microscopy

Powdered materials were cleared with sodium hydroxide and mounted in glycerin medium. After staining, different cell components were studied using compound microscope and microphotographs were taken with Nikon labphot 2 microscopic unit.

Physico – chemical evaluation

Ash values

Ash values such as total ash, water soluble ash, acid insoluble ash, sulphated ash were determined as per Indian Pharmacopoeia[21]. About 5 g of the root powder of *S. colais* and *S. suaveolens* were weighed and taken separately into a silica crucible, ignited up to 450°C and the total ash value was determined. The ash obtained was boiled with 25 mL of water and filtered through an ash less filter paper and the paper was ignited in a silica crucible, cooled and weighed. The water soluble ash was calculated by subtracting the water insoluble ash from the total ash. Similarly the ash obtained in the first step was boiled with 25 mL of dilute hydrochloric acid for 5 min and filtered through an ash less filter paper and the paper was ignited, cooled and the acid-insoluble ash value was calculated with respect to crude drug. About 5 g 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, cooled, weighed and the sulphated ash was calculated.

Extractive value

Ethanol soluble extractive and water soluble extractive values were determined as per Indian Pharmacopoeia[21]. About 5 g of dried coarse powder of roots of *S. colais* and *S. suaveolens* were macerated with 100 mL of 90% ethanol as well as water 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 as well as water soluble extractives were calculated with reference to air dried drug.

Crude fiber content

About 2 g of the powdered root of *S. colais* and *S. suaveolens* were defatted, boiled with sulphuric acid separately for 30 min followed by sodium hydroxide, and finally with water and alcohol. The residue was dried and incinerated. The percentage crude fiber content was calculated[21].

Loss on drying

A quantity of 1 g of powdered root of *S. colais* and *S. suaveolens* were taken separately 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[21].

Elemental analysis

The powdered root material of *S. colais* and *S. suaveolens* were taken and the minerals and heavy metals content were analysed using Atomic Absorption Spectroscopy.

Phytochemical evaluation

Preparation of extracts and determination of extractive values

The freshly collected root material of *S. colais* and *S. suaveolens* were cut into small pieces, shade dried and coarsely powdered. The powdered material was charged in an aspirator bottle and successively extracted with solvents such as petroleum ether, chloroform, ethyl acetate and ethanol by cold maceration for 72 h, 48 h and 24 h. After decantation and filtration, nearly 80% of the solvent was removed by distillation using rotary vacuum evaporator. The extracts obtained were further dried in vacuum desiccator and the yields of the extracts were calculated.

Fluorescence analysis

The powdered root material and various extracts of *S. colais* and *S. suaveolens* were subjected to fluorescence analysis in day and UV light[22].

Qualitative phytochemical evaluation

The powdered root material of *S. colais* and *S. suaveolens* were subjected to preliminininary phytochemical analysis[23,24].

Quantitative phytochemical evaluation

The secondary metabolites such as phenols, tannins (Singleton *et al.,* 1999)[25-26] and flavonoids (Zhishen *et al*., 1999)[27] were estimated by standard methods.

High performance thin layer chromatography

The extracts (5 µL) were applied separately on silica gel 60 F_{254} precoated HPTLC plates, 7 x 10 cm (Merck, Darmstadt, Germany) with the help of CAMAG automatic TLC sampler 4 (ATS 4) and eluted to a distance of 8 cm at room temperature (25°C) in the respective solvent system. Then the plates were developed in a CAMAG twin trough chamber (20×10 cm) pre-saturated with the respective mobile phase. The plates were heated at 60°C for 5 min. The plates were observed under UV- 254 nm and 366 nm light in CAMAG-UV cabinet and the HPTLC fluorescence image documented. The corresponding digital scanning profiling was carried out with a CAMAG-TLC scanner 3 equipped with win CATS planar chromatography manager software at a wavelength of 254 nm and 366 nm.

Results

Macroscopical evaluation

The young roots of *S. colais* and *S. suaveolens* were taken and the organoleptic and morphological features were observed (Fig. 1) *S. colais is* yellowish brown, characteristic odour, bitter and astringent taste, cylindrical in shape, fibrous fracture with fissures and lenticels on surface. *S. Suaveolens* is dark brown, characteristic odour, astringent taste, cylindrical shape, short fracture with deep fissures with transversely extended lenticels. The macroscopical observations are on par with monograph of Ayurvedic Pharmacopoeia of India (API).

Microscopical evaluation

Transverse section of root of S. colais

It consists of thin periderm, wide cortex, secondary phloem and central solid cylinder of secondary xylem. The periderm is superficial and it varies in thickness. Phellem is heterogenous comprised of 3 to 5 layers tubular and suberized cells. The single layer of thick walled and lignified 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, consists of dilated rays and thin discontinuous segments of fiber (20 µm dia) occur in circular cylinder in the phloem. The

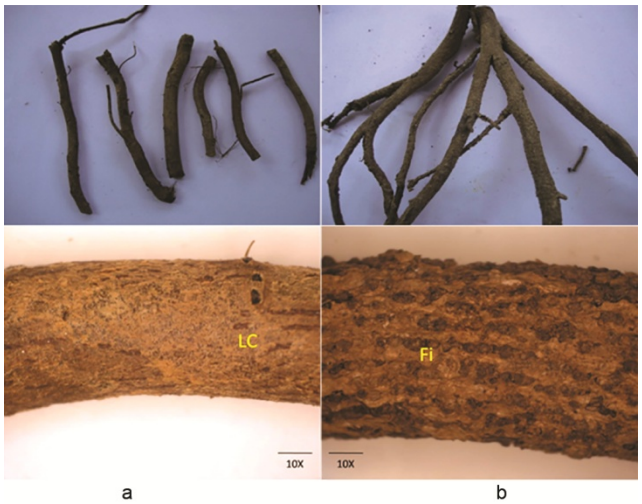


Fig. 1 — A&B Macroscopy of root of *S. colais* and *S. suaveolens* respectively, LC- lenticels, Fi-fissures

sieve elements are very small, angular in outline and have small companion cells at one corner of the cells. The phloem rays traverse the sclerenchyma cylinders. The secondary xylem cylinder is 900 µm in diameter. It includes diffusely distributed wide circular thin walled vessels and xylem fibres (Fig. 2A).

Transverse section of root of S. suaveolens

The root bark exhibits highly complex structure and organization. The periderm is of multiple types known as rhytidome. The first formed periderm is quite thick and highly undulate having deep furrows and thick ridges. The periderm is 400 µm thick, 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 have cellulosic cell wall. 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 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). Phloem fibres are scattered in the collapsed phloem (at par with API). The sieve elements are crushed into dark masses or lines. Secondary xylem occurs in thick, circular, compact cylinder. 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 ring, the vessels are narrow, less in frequency and thick walled (Fig. 2B).

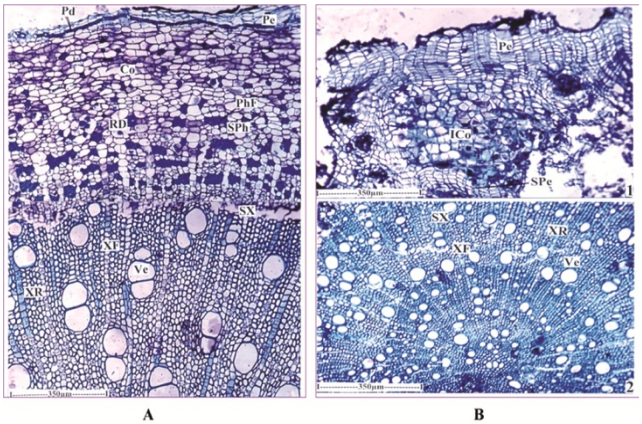


Fig. 2 — A&B: Transverse section of root of *S. colais* and *S. suaveolens*. (Co – Cortex, Pd – Phelloderm, Pe – Periderm, PhF – Phloem Fibres, RD – Ray dilation, SPh- Secondary Phloem, SX – Secondary Xylem, Ve – Vessel, XF – Xylem fibre, XR – Xylem Ray, ICo – Inner cortex, SPe – Sequent Periderm)

Transverse section of the root bark of S. colais

The bark is rough surfaced with minute fissures. It consists of a thin superficial periderm and wide cortex followed by secondary phloem. The cortical cells are large, tangentially elongated and compressed. The secondary phloem is differentiated into outer wide zone of collapsed phloem and inner narrow zone of non-collapsed phloem with a distinct boundary. The collapsed phloem is wider and includes several scattered masses of fibers (20 µm dia) and dilated rays. Fibers are absent in the non-collapsed phloem. The sieve elements are polygonal in outline, thick walled and have distinct companion cells (Fig. 3A).

Transverse section of root bark of S. suaveolens

The bark is rough surfaced with deep fissures. It consists of a rhytidome comprised of primary and sequent periderm. The cortical tissue or secondary phloem is enclosed within the loop of the sequent periderm. 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 fibers are diffusely distributed in the collapsed phloem (at par with API). (Fig. 3B).

Tangential longitudinal section (TLS) of the phloem of S. colais

In T.L.S view, the phloem rays appear non-storied and uniseriate, in part multiseriate or fully multiseriate. The rays have long upright cells and squarish procumbent cells. The rays are 200-400 µm in height and 30 µm in breadth. The phloem parenchyma cells are rectangular and vertically elongated. The phloem fibres are long and narrow,

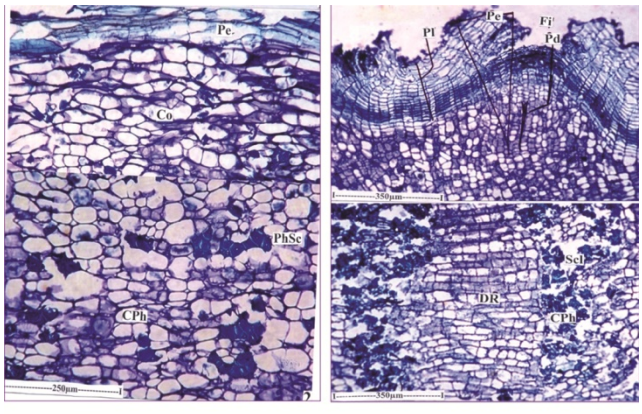


Fig. 3 — A&B: Transverse section of root bark of of *S. colais* and *S. suaveolens.* (Co – Cortex, CPh – Collapsed Phloem, Pe – Periderm, PhSc – Phloem Sclerenchyma, DR – Dilated ray, Fi – Fissure, Pd – Phelloderm, Pl – Phellem, Sc - Sclerenchyma)

thick walled with wide lumen (Fig. 4A). The vessels (70 µm dia) are either in solitary or in multiples of two. The xylem fibres have thin walls and wide lumen. The xylem rays are in thin straight radial lines (Fig. 4C.1).

Tangential longitudinal section (TLS) of the phloem of S. suaveolens

The phloem rays appear as uniseriate, biseriate to multiseriate (at par with API). The rays are up to 280-320 µm in height and 50 µm thick. The rays are heterocellular; the rays consist of middle portion of squarish or horizontally elongated procumbent cells at the end of rays (Fig. 4B). 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 fibers are thick walled, lignified and arranged in compact radial rows (Fig. 4C.2).

Crystal distribution

Calcium oxalate druses are occasionally seen in the collapsed phloem parenchyma cells of root of *S. colais* However, calcium oxalate crystals are fairly abundant in the cortical portion of the root of *S. suaveolens*. The crystals exist as raphides in the outer cortex which are thin, long pointed needles compactly bundled into spindle shaped bodies and druses in the inner cortex which are spherical bodies formed by the aggregation of compactly arranged, small pointed prismatic crystals.

Powder microscopy

The powder preparation of the root material of *S. colais* exhibits long, narrow, thick walled fibres with tapering ends. The fiber walls are very thick (20 µm) and the cell lumen is very narrow. Small fragments of homocellular periderm tissues are seen in surface view. The cells are radially elongated, compact and parallel to each other. The cell walls are suberized and thick walled There are free floating, spherical, dark red lipid bodies of different sizes when stained with sudan red. The *S. suaveolens* root powder exhibits narrow, thin walled fibers (on par with API). Small fragments of periderm cells with thick radial walls are seen in surface view. Spherical dark red lipid bodies are very frequent in the powder when stained with Sudan red.

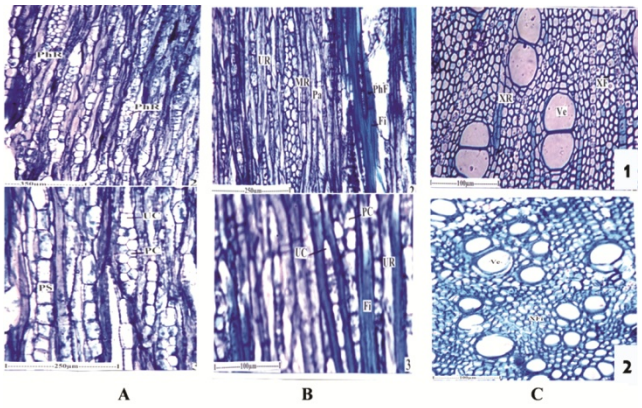


Fig. 4 — A, B: Tangential longitudinal section of phloem *S. colais* and *S. suaveolens* roots, C: 1, 2 - Secondary xylem of *S. colais* and *S. suaveolens* roots respectively. (PC – Procumbent cells, PR – Phloem Ray, PS – Parenchyma strand, UC – Upright Cell), (Fi – Fibre, MR – Multiseriate ray, Pa – Parenchyma, Pc – Procumbent cell, PhF – Phloem fibre, PhR – Phloem ray, Uc – Upright cell, UR – Uniseriate ray, Fi - Fibre)

Physico-chemical evaluation

Physico-chemical constants such as ash values, extractive values, crude fibre content, loss on drying of the roots of *S. colais* and *S. suaveolens* were determined as quality control parameters and tabulated in Table 1.

Elemental analysis

The amount of heavy metals such as lead, cadmium, mercury and arsenic were estimated in the roots of *S. colais* and *S. suaveolens*. The result revealed that the levels were within the limit of WHO standards[28] in both the species. The amount of other elements such as copper, zinc, sodium, potassium, selenium and iron was estimated and the results were tabulated (Table 2).

Phytochemical evaluation

Preparation of extracts and extractive value of the roots

The powdered root materials of *S. colais* and *S. suaveolens* were successively extracted with solvents such as petroleum ether, chloroform, ethyl acetate and ethanol by cold maceration.

Table 1 — Physico-chemical constants

S.No.	Parameters	Percentage w/w	
		S. colais	*S. suaveolens*
I.	Ash values		
1.	Total ash	2.54±0.12	6.83±0.29
2.	Acid insoluble ash	0.12±0.04	2.91±0.07
3.	Water soluble ash	1.13±0.43	2.25±0.62
4.	Sulphated ash	0.86±0.27	1.12±0.78
II.	Extractive values		
1.	Ethanol soluble extractive	4.81±0.18	2.17±0.09
2.	Water soluble extractive	5.63±0.32	4.15±0.26
III	Crude fibre content	1.27±0.24	1.69±0.12
IV	Loss on drying	8.10±2.43	7.98±1.98

Values are expressed as Mean ± SD of triplicates

Fluorescence analysis

The fluorescence character of powdered root material and various extracts of *S. colais* and *S. suaveolens* were studied in day and UV light). The pink colour with alcoholic potassium hydroxide reveals the presence of anthraquinones in *S. suaveolens.*

Qualitative phytochemical evaluation

Preliminary phytochemical analysis of the powdered root material of *S. colais* and *S. suaveolens* were carried out and recorded (Table 3. *S. suaveolens* showed the presence of terpenes and anthraquinones in addition to flavonoids, phenols and tannins as shown by both the species.

Quantitative phytochemical evaluation

The estimation of secondary metabolites in various extracts of *S. colais* and *S. suaveolens* were carried out and the results were tabulated (Table 4).

HPTLC finger print analysis

The HPTLC fingerprint profile of various extracts of roots of *S. colais* and *S. suaveolens* such as PESC-PESS (Fig. 5), CESC-CESS (Fig.6), EASC-EASS (Fig. 7), EESC-EESS (Fig. 8) were taken and the distribution of phytoconstituents were compared.

Discussion

The pharmacognostical parameters such as macroscopy, microscopy, physico-chemical analysis and elemental analysis were carried out to standardize the roots of *S. colais* and *S. suaveolens*. The root of *S. colais* is yellowish brown in colour, bitter and astringent in taste whereas, *S. suaveolens* is dark brown in colour and astringent in taste. The fissures observed in the outer surface are more and deep in *S. suaveolens* than in *S. colais*. In microscopical studies, heterocellular periderm is observed in *S. colais* and multitype (Rhytidome) periderm is observed in *S. suaveolens* which is in confirmation with the description of Ayurvedic Pharmacopoeia of India[18]. Calcium oxalate druses are observed in the collapsed phloem region of *S. colais* while in *S. suaveolens* the crystals exist as raphides in inner cortex and as druses in the outer cortex region.

Table 2 — Elemental composition of roots of *S. colais* & *S. suaveolens*

S. No	Name of the element	*Stereospermum colais*	*Stereospermum suaveolens*	WHO standards
1.	Lead	3.12 ppm	5.45 ppm	10 ppm
2.	Cadmium	0.19 ppm	0.24 ppm	1 ppm
3.	Mercury	Nil	0.012 ppm	0.5 ppm
4.	Arsenic	0.12 ppm	0.45 ppm	5 ppm
		Percentage w/w per 100 g		
5.	Copper	2.31 mg	1.22 mg	-
6.	Zinc	3.8926 mg	4.364 mg	-
7.	Sodium	63.6 mg	57.6 mg	-
8.	Potassium	89.4 mg	120.6 mg	-
9.	Selenium	2.35 µg	1.35 µg	-
10.	Iron	4.108 mg	13.47 mg	-

Table 3 — Preliminary phytochemical analysis of root powder of *S. colais* and *S. suaveolens*

S. No.	TEST	REAGENT	RESULT	
			S. colais	*S. suaveolens*
1.	Terpenes	Tin + thionyl chloride	-	+
2.	Flavonoids	Mg turnings + Conc. HCl	+	+
3.	Steroids	Acetic anhydride + H_2SO_4	+	+
4.	Carbohydrate	Fehling's I & II	+	+
5.	Glycosides	Anthrone + H_2SO_4	+	+
6.	Quinones	NaOH	+	+
7.	Alkaloids	Dragendorff's reagent	-	-
8.	Phenols	Ferric chloride	+	+
9.	Tannins	Lead acetate	+	+
10.	Saponins	Water	+	+
11.	Proteins	Picric acid	+	+
12.	Anthraquinones	Ammonia	-	+

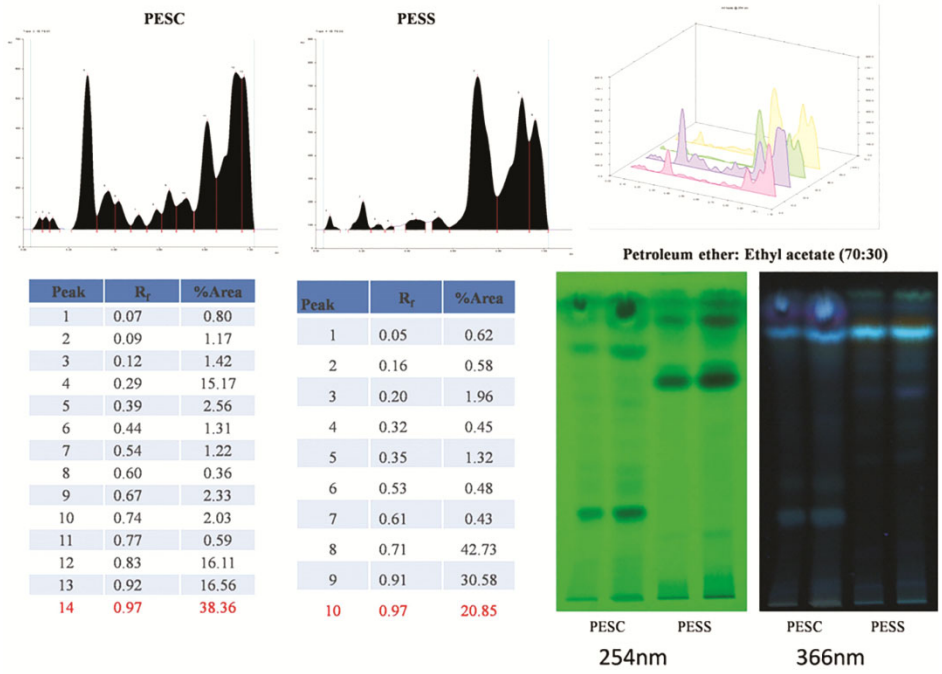
(+) Present (-) Absent

Table 4 — Quantitative estimation of primary and secondary metabolites of various extracts of the roots of *S. colais* and *S. suaveolens*

Metabolites	Amount of metabolites (mg/gm)			
	Stereospermum colais		*Stereospermum suaveolens*	
	EASC	EESC	EASS	EESS
Phenols (Gallic acid equivalent)	13.93±3.83	8.3±1.67	13.95±2.08	6.5±1.29
Tannins (Tannic acid equivalent)	-	26.66±0.49	-	36.77±2.98
Flavonoids (Quercetin equivalent)	45.06±3.56	22.04±2.99	49.82±2.86	46.16±3.41

Values are expressed as Mean ± SD of triplicates

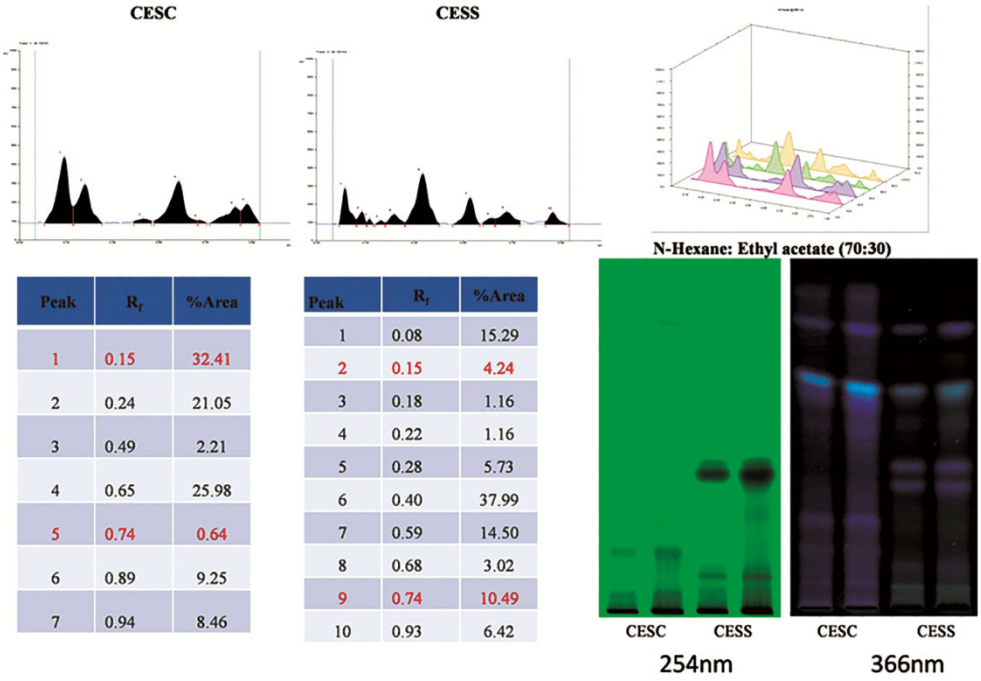
(PESC – Petroleum ether Extract, CESC – Chloroform Extract, EASC – Ethyl Acetate Extract, EESC – Ethanol Extract of *S. colais,* PESS – Petroleum ether Extract, CESS – Chloroform Extract, EASS – Ethyl Acetate Extract of, EESS – Ethanol Extract of *S. suaveolens*)



Peak	R_f	%Area
1	0.07	0.80
2	0.09	1.17
3	0.12	1.42
4	0.29	15.17
5	0.39	2.56
6	0.44	1.31
7	0.54	1.22
8	0.60	0.36
9	0.67	2.33
10	0.74	2.03
11	0.77	0.59
12	0.83	16.11
13	0.92	16.56
14	0.97	38.36

Peak	R_f	%Area
1	0.05	0.62
2	0.16	0.58
3	0.20	1.96
4	0.32	0.45
5	0.35	1.32
6	0.53	0.48
7	0.61	0.43
8	0.71	42.73
9	0.91	30.58
10	0.97	20.85

Fig. 5 — HPTLC fingerprint of petroleum ether extract of *S. colais* and *S. Suaveolens*
(PESC – Petroleum ether extract of *S. colais,* PESS – Petroleum ether extract of *S. suaveolens*)

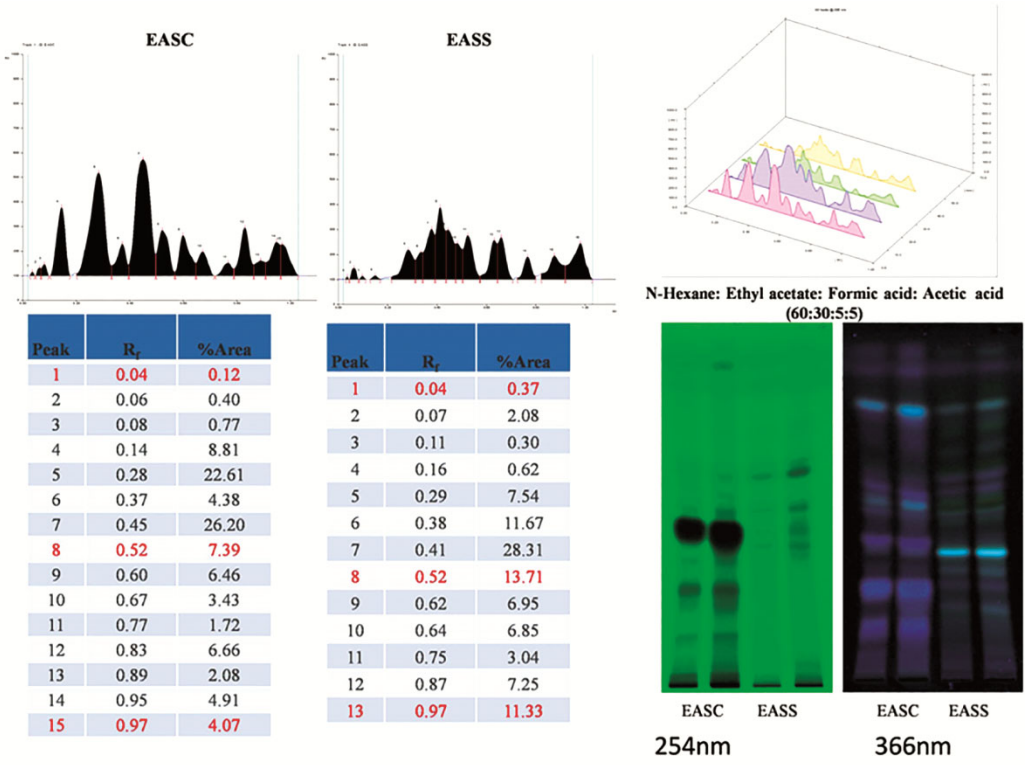


Peak	R_f	%Area
1	0.15	32.41
2	0.24	21.05
3	0.49	2.21
4	0.65	25.98
5	0.74	0.64
6	0.89	9.25
7	0.94	8.46

Peak	R_f	%Area
1	0.08	15.29
2	0.15	4.24
3	0.18	1.16
4	0.22	1.16
5	0.28	5.73
6	0.40	37.99
7	0.59	14.50
8	0.68	3.02
9	0.74	10.49
10	0.93	6.42

Fig. 6 — HPTLC fingerprint of chloroform extract of *S. colais* and *S. suaveolens*
(CESC – Chloroform Extract of *S. colais,* CESS – Chloroform Extract of *S. suaveolens*)

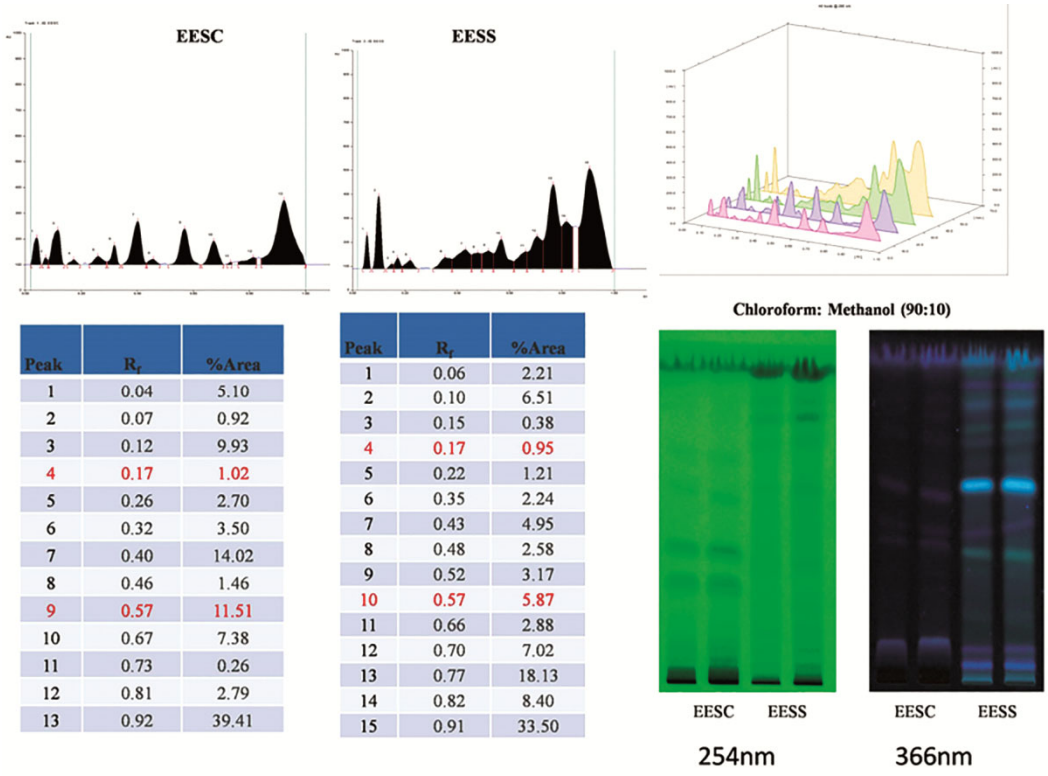
deep in *S. suaveolens* than in *S. colais*. In microscopical studies, heterocellular periderm is observed in *S. colais* and multitype (Rhytidome) periderm is observed in *S. suaveolens* which is in confirmation with the description of Ayurvedic Pharmacopoeia of India[18]. Calcium oxalate druses are observed in the collapsed phloem region of *S. colais* while in *S. suaveolens* the crystals exist as raphides in inner cortex and as druses in the outer cortex region.



Peak	R_f	%Area
1	0.04	0.12
2	0.06	0.40
3	0.08	0.77
4	0.14	8.81
5	0.28	22.61
6	0.37	4.38
7	0.45	26.20
8	0.52	7.39
9	0.60	6.46
10	0.67	3.43
11	0.77	1.72
12	0.83	6.66
13	0.89	2.08
14	0.95	4.91
15	0.97	4.07

Peak	R_f	%Area
1	0.04	0.37
2	0.07	2.08
3	0.11	0.30
4	0.16	0.62
5	0.29	7.54
6	0.38	11.67
7	0.41	28.31
8	0.52	13.71
9	0.62	6.95
10	0.64	6.85
11	0.75	3.04
12	0.87	7.25
13	0.97	11.33

Fig. 7 — HPTLC fingerprint of ethyl acetate extract of *S. colais* and *S. suaveolens*
(EASC – Ethyl Acetate extract of *S. colais,* EASS – Ethyl Acetate extract of *S. suaveolens)*



Peak	R_f	%Area
1	0.04	5.10
2	0.07	0.92
3	0.12	9.93
4	0.17	1.02
5	0.26	2.70
6	0.32	3.50
7	0.40	14.02
8	0.46	1.46
9	0.57	11.51
10	0.67	7.38
11	0.73	0.26
12	0.81	2.79
13	0.92	39.41

Peak	R_f	%Area
1	0.06	2.21
2	0.10	6.51
3	0.15	0.38
4	0.17	0.95
5	0.22	1.21
6	0.35	2.24
7	0.43	4.95
8	0.48	2.58
9	0.52	3.17
10	0.57	5.87
11	0.66	2.88
12	0.70	7.02
13	0.77	18.13
14	0.82	8.40
15	0.91	33.50

Fig. 8 — HPTLC fingerprint of ethanolic extract of *S. colais* and *S. suaveolens*
(EESC – Ethanol Extract of *S. colais*, EESS – Ethanol Extract of *S. suaveolens*)

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration also to establish appropriate standards. Physico-chemical analysis showed that the ash value was found to be more in *S. suaveolens* (6.83% - [API value: not more than 8%]) than in *S. colais* (2.54% [API value: not more than 6%]) thereby showing more inorganic elements in *S. suaveolens*, the results corroborate with the Ayurvedic Pharmacopoeia of India reference values[18]. The extractive values were found to be more in *S. colais* (ethanol and water soluble extractives 4.81% and 5.63% respectively) than *S. suaveolens* (2.17% and 4.15% respectively [(API value: not less than10 and 20 respectively)] showing the presence of more non polar compounds in *S. colais* than *S. suaveolens* in corroboration with the presence of non-polar compounds such as terpenes and anthraquinones in *S. suaveolens* (Table 3) and the higher percentage of flavonoids (Table 4). 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 roots[28]. When analyzing the other elemental composition of the roots, copper, sodium and selenium content was found to be more in *S. colais* while *S. suaveolens* was found to be rich in zinc, potassium and iron content.

The fluorescence character of powdered root materials and various extracts of *S. colais* and *S. suaveolens* were marginally different from each other. The powdered plant material of *S. colais* showed the presence of steroids, flavonoids, quinones, phenols, tannins, glycosides, carbohydrates, saponins, proteins and aminoacids. The powdered plant material of *S. suaveolens* showed the presence of terpenoids, anthraquinones, steroids, flavonoids, quinones, phenols, tannins, glycosides, carbohydrates, saponins, proteins and aminoacids (Table 3). Both the plants differ in the presence of terpenoids and anthraquinones which were present in *S. suaveolens* and absent in *S. colais*. The phytochemical screening of various extracts of *S. colais* and *S. suaveolens* were studied and reported by the authors and the results were divergent with respect to each extract[11]. The secondary metabolites such as phenols, tannins and flavonoids are responsible for the pharmacological activity of the plant. The amount of secondary metabolites such as phenols, tannins and flavonoids were found to be marginally high in *S. suaveolens* when compared to *S. colais*.

HPTLC is an economical method for separation, qualitative identification or semi-quantitative analysis of samples and it can be used to solve many qualitative and quantitative analytical problems. HPTLC finger printing is used as a tool for standardization and validation of the two species and helps the industry to authenticate the raw material before taking it to the formulation. HPTLC fingerprints of various extracts of roots of *S. colais* and *S. suaveolens* were taken and comparative study was made. The profiles exhibit the presence of similar compounds and perhaps as a diagnostic tool for the identification of the plant material in future. HPTLC profile of petroleum ether extracts showed 14 and 10 polyvalent peaks in PESC and PESS respectively, in which one common peak was observed at the R_f value of 0.29 (Fig. 5). 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 (Fig. 6. 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_f value of 0.04, 0.52 and 0.97 (Fig. 7). 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_f value of 0.17 and 0.57 (Fig. 8).

Conclusion

The plants selected for this study, *S. colais* and *S. suaveolens* are named as "Patala" in Ayurvedic medicine. The roots of "Patala" have rich traditional value especially in the treatment of inflammation and rheumatism besides one of the ingredients in Dasamula. Even though "Patala" is a reputed Ayurvedic plant used for various ailments, there is a lacuna in the authentication and identification of the roots of two *Stereospermum* species. The results obtained from the comparative pharmacognostical and phytochemical studies of the roots will help in identification and authentication of the two species. The standardization parameters that have been developed here can be used as reference standard for correct identification of "Patala" in future and also useful in making a monograph of the plant. Further, it acts as a tool to detect adulterants and substituent and consequently maintains the quality, reproducibility and efficacy of the two *Stereospermum* species.

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Conflict of Interest

The authors do not have conflict of interest

Authors' Contributions

LS: Conceptualization; Formal analysis**,** writing original draft; SS: Supervision; CD: Conceptualization; Writing - review & editing; and SR: Supervision

References

1 Verma S & Singh S P, Current and future status of herbal medicines, *Vet World*, 1 (11) (2008) 347-350.

2 Dineshkumar C, Pharmacognosy can help minimize accidental misuse of herbal medicine, *Curr Sci*, 3 (2007) 1356-1358.

3 Sumitra C, Importance of pharmacognostic study of medicinal plants: An overview, *J Pharmacogn Phytochem*, 2 (5) (2014) 69-73.

4 Chopra R N, Nayar S L & Chopra I C, Glossary of Indian Medicinal Plants, (Council of Scientific and Industrial Research, New Delhi), 1956, 235.

5 Kritikar K R & Basu B D, Indian Medicinal plants, Vol.2, (International book distributors, Dehradun), 1999, p. 1848.

6 Meena A K, Yadav A K, Panda P, Preet, K & Rao M M, Review on *Stereospermum suaveolens* DC: A potential herb, *Drug Invent Today*, 2 (5) (2010) 238-239.

7 Balasubramanian T & Chatterjee T K, Analgesic and antipyretic activities of ethanol extract of *Stereospermum suaveolens*, *J Diet Suppl*, 7 (2) (2010)a 104-116.

8 Balasubramanian T, Tapan Kumar C, Sarkar M & Meena S L, Anti-inflammatory effect of ethanol extract of *Stereospermum suaveolens* in rats, *Pharm Biol*, 48 (3) (2010)b 318-323.

9 Kharat U, Chanshetti R, Chavan, V, Naik, Y & Date N, Evaluation of anti-inflammatory potential of aqueous extract of root bark of *Stereospermum suaveolens* DC., *Int J Pharm Pharm Sci*, 4 (3) (2012) 494-496.

10 Mrinmoy N, Pulok K M, Joydeb C, Rajarshi B, Ranjit K H, *et al.,* Plant developed analytical profile of *Stereospermum suaveolens* in Indian Traditional Knowledge, *Indian J Tradit Know*, 14 (4) (2015) 590-594

11 Latha S, Seethalakshmi S, Chamundeeswari D, Senthamarai R, Shanthi S, *et al.,* Comparative phytochemical and antioxidant studies on roots of *Stereospermum colais* & *Stereospermum suaveolens*, *Int J Phytopharm*, 5 (4) (2014) 301-305.

12 Latha S, Chamundeeswari D, Seethalakshmi S & Senthamarai R, Attenuation of adjuvant-induced arthrits by *Stereospermum colais* and *Stereospermum suaveolens* via modulation of inflammatory mediators, *J Ethnopharmacol,* 1 (249) (2020) 112394.

13 Chunekar K C, Bhavaprakasa nighandu by Sri Bhavamisra, (Chaukhamba Sanskrit Sansthan, Varanasi, India), 1982, p. 279-280.

14 Nadakarni A M & Nadkarani K M, Indian Materia Medica, (Popular Prakashan, Bombay), 1954.

15 Kapoor S L & Mitra R, Herbal drugs of Indian pharmaceutical Industry, (National Botanical Research Institute, Lucknow), 1979, p. 66.

16 Kurup P N V, Ramdas V N K & Joshi P, Handbook of Medicinal Plants, (Central Council for Research in Ayurveda and Sidda, New Delhi), 1979, 164.

17 Manilal K S, Van Rheede's Hortus Malabaricus (Malabar garden) english edition with annotations and modern botanical nomenculature, (University of Kerala, Thiruvananthapuram), 2003.

18 Anonymus, The Ayurvedic Pharmacopoeia of India, 1st edition, Part 1, Vol 3, Delhi, (Government of India, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine & Homoeopathy), 2001.

19 Johansen D A, *Plant Microtechnique*, (Mc Graw Hill Book Co, New York), 1940, p. 523.

20 O'Brien T P, Feder N & McCull ME, Polychromatic staining of plant cell walls by toluidine blue-O, *Protoplasma,* 59 (1964) 364-373.

21 Anonymous, Indian Pharmacopoeia, Vol. 2, Ministry of Health and Family Welfare, (Government of India, The Controller of Publications, New Delhi), 1996.

22 Chase C R & Pratt R S, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J Am Pharm Assoc*, 38 (1949) 384-331.

23 Madhu C D, Plant Drug Evaluation: Laboratory guide, 2nd edn. (Cd remedies, Coimbatore), 2002.

24 Pulok M K, Quality Control of Herbal Drugs – An Approach to Evaluation of Botanicals, (Business Horizons pharmaceutical publishers, New Delhi), 2002.

25 Singleton V L, Orthofer R & Lamuela R R M, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Methods Enzymol,* 299 (1999) 152-179.

26 Zhishen J, Mengcheng T & Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem*, 64 (1999) 555-559.

27 Rashmi S, Neha M & Neetu M, Evaluation of the extraction hours and solvent concentrations on secondary metabolites and antioxidant activity of *Feronia limonia* fruit, *Indian J Nat Prod Resour,* 12 (3) (2021) 463-471.

28 Anonymus, WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues, (World Health Organization, Geneva, Switzerland), 2006.