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Pharmacognostical and preliminary phytochemical properties of *Stereospermum chelonoides* (L.F.) DC Mixed preparation of Flower and Leaf

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Abstract
The anatomical, pharmacognostical and preliminary phytochemical studies of leaf and flower powder mixture of *Stereospermum chelonoides* were studied to understand the quality of botanical raw materials used in health industry. Morphological features, microscopic characters and physiochemical features like total ash, acid insoluble ash, water soluble ash, organic solvent extractives, phytochemical nature of the powder and extracts, fluorescence nature of the powder and microbial limit assay were performed using standard textual methods. The morphological, microscopical features and microbial nature were within the limits of ayurvedic pharmacopeia of India. Extracts and powder showed the presence of terpenoids, flavonoids, Saponins, phenolic compounds and carbohydrates. These observations would be of immense value in the botanical identification and standardization of drugs in a crude form.

Keywords: *Stereospermum chelonoides*, phytochemical characters, pharmacognostic parameters

Introduction
Indian system of medicine uses medicinal plants as a raw material and processed it for on the basis of pharmacological formulations. Now-a-days a good number of people renewed their interest towards the usage of drugs of herbal origin due to harmful nature of the synthetic drugs. The safety of the herbal medicine has turned to be a concern due to non-availability of herbs to meet the rising demand and adulterated with low quality or similar plant materials. Adulterated medicinal plants could reduce therapeutic efficacy as it primarily depends on the quality of raw materials [1]. Similar vernacular names given to multiple plants could be solved by anatomic study, powder analysis, pharmacognostic and physicochemical analysis, whereby the AYUSH also insisted the importance of pharmacognostic specifications of medicinal plants. In view of the above, pharmacognosy deals with standardization and authentication studies of natural drugs [2]. Traditional healers used the leaves of this plant to treat otalgia, odantalgia, rheumatalgia, malarial fever, wounds and acts as antipyretic agent. It also used to treat chronic dyspepsia [3]. Flowers are used to treated burning sensation of urination, able to cure pitta and vata, heart problems and cough. Decoction of the flowers mixed with honey used for the treatment of cough [4]. An infusion prepared from the root and flowers used for the treatment of fevers. The juice of the bark is used to treat indigestion. Flowers are used in bleeding disease, sore throat and diarrhea; fruits are useful in blood diseases. Different parts of the tree are used for pharmacological purposes [5]. Outcomes lay down standardization parameter to identify and prevent adulterations in medicinal plant health care products even in dried powdered form. In this context, pharmacognostic studies are mandatory because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. Such studies would assist in the authentication of medically potential plants and would ensure reproducible quality of herbal products leading to safety and efficacy of natural products.

Materials and methods
Collection of Plant Material
The flower and leaf of *Stereospermum chelonoides* (L.F.) DC were collected from the Thyagaraja Swamy Temple in Thiruvarur, Tamil Nadu and plant material was authenticated by Dr. P. Prabakaran, Professor M. R. Government Arts College, Mannargudi. The flower and leaf were shade dried and then powdered using a mechanical grinder.

Microscopy-anatomy
Plant materials were sectioned using Rotary Microtome, stained by toludine blue, safranin, fast green and IKI and subjected to microscopic observations. Glycerin mounted specimens were observed and photomicrographs were taken using Nikonlab photo 2 microscopic unit. Bright field and polarized ligh were employed to take a photograph [6].

Powdered microscopy
Stomatal morphology, venation pattern, trichomes, crystals and other parts present in the powder were using powder microscopy. Powder materials were placed on slides and mounted with 2-3 drops of chloral hydrate and each slide was covered with cover slip then examined under microscope. Different cell components were noted and photography was done by using digital camera [7].

Organoleptic Evaluation
Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste, texture, etc. The organoleptic characters of the samples were evaluated based on the textual methods [8].

Physicochemical Parameters
Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matter were analyzed by the methods given in the ayurvedic Pharmacopoeia of India [9].

Fluorescence Analysis
Leaf and flower mixed powder of *Stereospermum chelonoides* were analyzed for its fluorescence parameters under day light as well as in ultra violet and observed after 48 hours of treatment with various chemical and organic solvents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid [10, 11].

Microbial Limit Assay
Aerobic bacterial and fungal elements were analysed by making use of 1 gm of leaf and flower mixed powdered preparation. Plant material was serially diluted using sterile saline as diluent. Nutrient agar medium, Rose Bengal agar and SS agar were used for isolating bacteria, fungi and enteric pathogens respectively. For bacteria, the plates were incubated at 37°C for 48 hrs and for fungi; the plates were incubated 25°C for 96 hrs [7].

Qualitative Phytochemical Screening
Freshly prepared leaf and flower mixed powder preparation of *Stereospermum chelonoides* were tested for the presence of phytochemical constituents using standard methods [12].

Results
Good and appropriate raw materials are needed for any treatment. Pharmacognostic standard determined in this study are used in determining the quality of raw material used for the assessment. Standardized raw materials are used for all type of pharmacological study and TSM. Now a day people concerned with the quality of raw materials used for the treatment as few drugs are available in the markek with different vernacular name. Hence, in the present investigation efforts were made to provide scientific data to determine standards for the plant materials. The present study on microscopical features and other physicochemical, phytochemical and fluorescence parameters on the flower and leaf mixed preparation of *Stereospermum chelonoides* will be useful in identifying the correct species of the plant.

Leaf anatomy
The leaf consists of thin lamina and thick midribs. The midrib is slightly convex on the AdH side and broad and thick on the AbX side. The midrib measures 1.2 mm in vertical plane and 1.15 mm in horizontal plane. The epidermal cells of the midrib, both on the adaxial and abaxial sides are semi-circular with slightly echinate outer radial walls. The ground tissue of the midrib consists of outer five layers of collenchyma cells and inner six layers of compact parenchyma cells. The vascular system has an adaxial thick straight segment of vascular strand and abaxial are of vascular strand. The vascular strands are collateral with inner xylem and outer phloem. It contains wide circular xylem elements and thick-walled vessels and thick walled, liquefied fileses. Phloem elements are in thick isolated masses located beneath each xylem segments (Figure 1).

Flower anatomy
In longitudinal sectional view (L.S. View), flower shows thick circular disc at the base of the ovary. The ovary in bicapillary syncarpous with many ovules on axial peculations. The sepal and petal arise from the base of the ovary. The sepal has several ridges which consist of small vascular bundles. The marginal part of the sepal in curved and is blunt. The petals are repeatedly folded with in the calyx. The anther is dithecous and four chambered (Figure 1).

Powder Microscopy
Epidermal trichomes of both glandular and non-glandular types were found to the abundant in the powder. The trichomes occur on the sepals, petals and leaves. The trichomes are of the major types. Glandular trichomes are multicellular, uniseriate and unbranched. The cells of the trichomes are vertically elongated and thin walled. The glandular trichomes are 100-350µm long. Like glandular trichome, Non glandular trichomes are also equally abundant in the powder. These trichomes are multicellular, uniseriate and branched. The cells are rectangular and elongated. The epidermal peeling of AbX of the lamina shows stomata and epidermal cells. Stomata are dense and random in orientation. They are paracytic type. The stoma has two subsidiary cells on an either side of the stoma. Pollen grains are seen in abundance in the powder. The pollen grains are in group of light grain cell compactly arranged. The pollen grain has prominent eclinate exine. The pollen masses are 20um in diameter (Figure 1).

Organoleptic Characters
Processed flower and leaf powder of *Stereospermum chelonoides* was assessed for its organoleptic characters, it revealed that the powder was greenish yellow in colour, characteristic odour, bitter taste and rough texture (Table 1). When powder was analysed for its physicochemical parameters, it showed 5.8% total ash content followed by 2.4% water soluble ash and 1.6% acid insoluble ash. This is within the limits of ayurvedic pharmacopeia of India (Table 5.2). Extractive phytocompounds play a vital role in biological potentials. Mixed powder preparation showed 13.6% water extractives followed by 8.3% ethanol extractive, 4.5% chloroform extractive, 3.8% ethyl acetate extractive and 1.3% hexane extractives. Higher water extractive indicated that the presence of polar phytochemicals in the powder. This was done successively with increased polar solvents. This is also in line with ayurvedic pharmacopoeia of India.

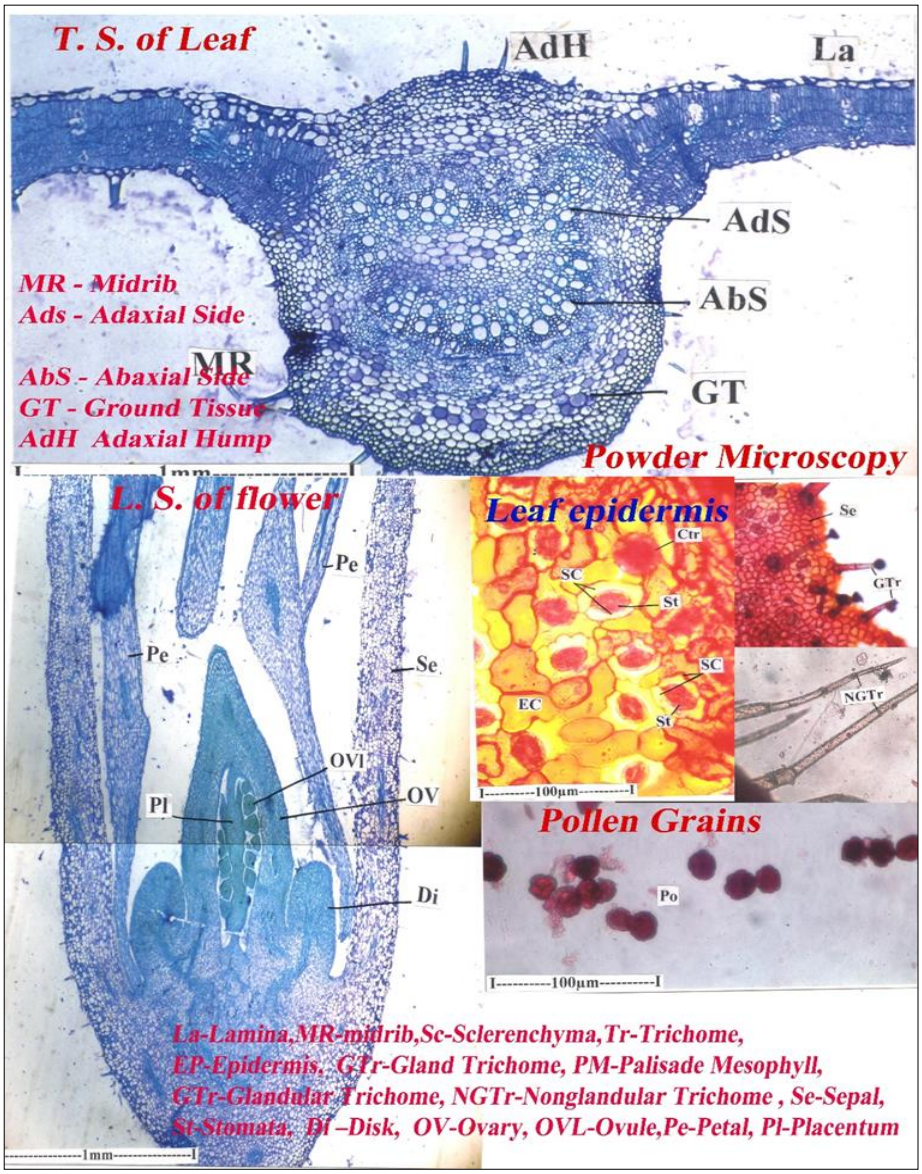

Fig 1: Anatomy and Powder microscopy of Stereospermumv chelonoides (L.F) DC

Table 1: Organoleptic characters of mixed powder preparation of *Stereospermum chelonoides*

S. No	Character	Observation/ Result
1	Colour	Greenish Yellow
2	Odour	Characteristic
3	Taste	Bitter
4	Texture	Rough

Table 2: Physicochemical constant and extractives of mixed powder preparation of *Stereospermum chelonoides*

S. No	Parameter	Results
1	Foreign matter	Nil
2	Total ash	05.80%
3	Acid insoluble ash	01.6%
4	Water soluble ash	02.40%
5	Water extractive	13.60%
6	Alcohol extractive	08.30%
7	Chloroform extractive	04.50%
8	Ethyl acetate extractive	03.80%
9	Hexane extractive	01.30%

Fluorescence analysis

The fluorescent feature of plant material plays a vital role in determining phytoconstituents in herbal drugs. It also provides the interaction or organic plant phytochemicals with inorganic and organic solvents. The leaf and flowe mixed powder was treated with various solvents and variable colour change with the formation of yellow, green, red, brown colouration under UV and VIS light. Mixed powder preparation of *Stereospermum chelonoides* showed characteristic brown, light brown, green, yellow, yellowish green, brown and black colour (Table 3). This indicated that different chromophores are available in the mixed powder preparation of *Stereospermum chelonoides*

Table 3: Fluorescence Analysis of mixed powder preparation of *Stereospermum chelonoides*

S. No	Test	Day light	UV light
1	Plant Powder + Chloroform	Light Brown	Light brown
2	Plant Powder + Hexane	Brown	Yellow
3	Plant Powder + Benzene	Brown	Brown
4	Plant Powder + Aqueous NaOH	Red	Red
5	Plant Powder + alcoholic NaOH	Yellow	Yellow
6	Plant Powder + 1N HCl	Brown	Yellow
7	Plant Powder + Ethanol	Light brown	Yellow
8	Plant Powder + Ethyl acetate	Reddish black	Black
9	Plant Powder + Acetone	Light brown	Yellow
10	Plant Powder + 50% H_2So_4	Brown	Black

Microbial limit assay

One of the important parameters in quality control of raw drugs and its powder is the assessment of microorganisms. Standard protocol was followed to detect microbial content of the powder. Results revealed that, the powder showed only $24x10^2$ CFU/g of total count of aerobic bacteria and 6CFU/g of total fungus. *Escherichia coli*, *Salmonella* and *Shigella* like enteric bacteria were completely absent in mixed powder preparation (Table 5.4). This is also within the limits of ayurvedic pharmacopoeia of India.

Table 4: Nature of microbial availability in mixed powder preparation of *Stereospermum chelonoides*

S. No	Test organism	Microbial count
1	Total aerobic Bacteria	24 x 10^2 CFU/g
2	Total Fungal count	06 CFU/g
3	Total Enteric Bacteria	Nil
4	Total *E. coli*	Nil
5	*Salmonella*	Nil
6	*Shigella*	Nil

Phytochemical Analysis

The results of preliminary quantitative phytochemical screening of mixed powder preparation of *Stereospermum chelonoides* revealed the presence of multiple polar and non-polar chemical constituents (Table 5). Saponins, Tannins, Flavonoids, coumarins, glycosides, sugar and phenolic compounds were present in powder preparations. Steroids are completely absent. Terpenoids are also indicated absent in powder.

Table 5: Preliminary Phytochemical screening of mixed powder preparation of *Stereospermum chelonoides*

S. No	Test	Result
1	Saponin	+ve
2	Tannin	+ve
3	Steroids	-ve
4	Terpenoids	-ve
5	Flavonoid	+ve
6	Coumarin	+ve
7	Alkaloids	-ve
8	Glycosides	+ve
9	Sugar	+ve
10	Phenols	+ve

Discussion

The present study revealed anatomical, pharmacognostic and phytochemical nature of SCFLM. It provides authentic information to check the nature of leaf and flower of *Stereospermum chelonoides*. It is also used to differentiate the powder from the closely related species. Number of anatomical and pharmacognostical nature of this plant and related genus are limited and lot of confusion in the differentiation of closely related species, this study would be useful the scientists those who are working in herbal medicine to develop authentication data for powdered drug from this plant. Pharmacognostic standards were determined through this study, it is also useful in detecting adulterations. Ash content was detected in low quantities confirmed the purity of the plant powder preparations, the availability of inorganic impurities like soil impurities were detected via ash values with reference to water soluble and acid insoluble ash value [10]. Results of the present study showed lower ash value, which indicated that the raw drug is free from foreign matter. The extractive values of the powdered plant materials indirectly illustrated the presence of specific polar or non-polar phyto constituents of the plant material [13]. Behaviour of drug materials under UV radiation and visible light exhibited different colour depending up on the various chromophores present in the material. The same extract may appear different at different wavelength of light [10, 11]. Flavonoids, protein, carbohydrates were the reason for higher water extractive. Hexane extractable value indicated the presence of straight chain fatty acid, which showed antimicrobial potentials of this plant mixed powder preparations. Phytochemical test of crude extract of this plant revealed the presence of flavonoid, alkaloid, quinones, cardiac glycosides, terpenoids, tannins and triterpenoids. Stereochenols A and B are the two Quinones isolated from *Stereospermum chelonoides* [14, 15] possess multiple potentials like antimicrobial and antioxidant activity. They also reported the presence of flavonoid, phenolic and tannin compounds. Iloki-Assanga et al., [16] stated the presence of about 2.964 µg/ml of flavonoid in aqueous extract of this plant [16]. The presence of flavonoid in mixed leaf and flower also indicated in UV spectra analysis [17]. Presence of phenolic compounds and tannins in plant materials are useful in antidiarrheal activity and antimicrobial activity, which was in agreement with various earlier reports [18, 19, 207]. The results of preliminary phytochemical analysis revealed the presence of alkaloids, terpenoids, flavanoids, tannins, saponins and carbohydrates in aqueous extract. Preliminary phytochemical tests were helpful in predicting the nature of drug and also useful for the detection of different constituent's present. Phenolic compounds present in the plant material are used in the treatment of burns as they precipitate the proteins of exposed tissue to form a protective covering [21]. They are also used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote [22]. Microbial limit assay indicated quality of raw or crude drug powder, number of microbial cells are directly proportional to the quality of the plant materials. This study provides quality parameters prescribed in Ayurvedic Pharmacopeia of India. This work provides qualitative and quantitative standards for the identification and authentication of raw plant powder.

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