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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STANDARDIZATION OF TUBEROUS ROOT OF *SMILAX CHINA* L.

B. Kumudhaveni * and R. Radha

The Tamil Nadu Dr. M. G. R Medical University, Guindy, Chennai - 600032, Tamil Nadu, India.

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Correspondence to Author:
B. Kumudhaveni

Research Scholar,
Department of Pharmacognosy,
College of Pharmacy, Madras Medical College, Chennai - 600003, Tamil Nadu, India.

E-mail: kumudhaveni@rediffmail.com

ABSTRACT: In this study, the pharmacognostic and phytochemical standardization of tuberous roots of *Smilax china* L. belongs to the Family Liliaceae was performed in detail. Pharmacognostical standardizations like macroscopical and microscopical studies, powder microscopy, quantitative microscopy, physicochemical analysis, fluorescence study, determination of heavy metal contamination, qualitative and quantitative estimation of inorganic elements, microbial contamination and preliminary phytochemical screening were performed as per WHO guidelines and other standard methods. The pharmacognostic evaluation was performed in order to create a standard monograph of the plant for identification as well as to detect the adulterants. Macroscopical study of tuberous root showed irregular, cylindrical, curved in shape and brownish-yellow in color. A microscopical study showed cortex, sclerenchyma, elongated parenchyma, starch grains, vascular bundles, fibers and tannin cells. Linear measurements were measured. Physicochemical analysis like ash value (Total ash - 0.5% w/w, acid insoluble ash – 0.06% w/w, water-soluble ash – 0.01% w/w, sulphated ash – 0.08% w/w), extractive value (water-soluble -16% w/w, alcohol soluble - 6% w/w), loss on drying (4.76% w/w), foaming index and fluorescence study were determined. Heavy metals and microbial contamination were determined. Iron was estimated as 4.44% w/w. Phytochemical screening showed the presence of alkaloids, carbohydrates, saponins, terpenoids, phenolic compounds, and tannins. Evaluation of tuberous root *Smilax china* L. ensures the identity and ascertains the quality and purity of this drug. The phytochemical study helps for the isolation and characterization of phytoconstituents present in this plant.

INTRODUCTION: Medicinal plants are traditionally used to treat various diseases for centuries throughout the world. Each traditional system has its own method for treating diseases. Nowadays, the usage of plant material as home remedies is increasing worldwide. Over the counter sale, supply of raw materials to the pharmaceutical industry, supply of drug products and raw materials to the world herbal drug market also increased. Hence, it is of utmost importance for identification, quality, and purity of herbal drugs.

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There are about 350 species of *Smilax* present in the tropical and temperate regions of the world, mainly in East Asia and North America [1]. *S. china* L. known as 'Jin Gang Teng' is indigenous to China and Japan, used traditionally in Chinese medicine. The English name is China root. *S. china* appears as large as a child's hand, twisted with full of knots, reddish on the outside, flesh-colored in the heart, and deprived of smell. It is used to treat diuretic, rheumatoid arthritis, gout, tumors, diabetes mellitus, laxative, epilepsy, inflammation, cancer and antibacterial [2-4]. A flavonoid glycoside, kaempferol - 7 - O - β – D - glucoside, triflavonoid kandelin, quercitin were isolated [5-8].

Antioxidant, hypoglycemic, hepatotoxicity, anti-convulsant activity was reported [9-12]. This drug is imported to the Indian market. The present study is

aimed to standardize the tuberous root of *Smilax china* L. belongs to the family Liliaceae to ensure the drug for its quality and more efficacies.

Taxonomy of *S. china:*

Kingdom	**:**	Plantae
Subkingdom	**:**	Tracheobionta
Super division	**:**	*Sperrmatophyta*
Division	**:**	Magnoliophyta
Class	**:**	Liliopsida
Subclass	**:**	Lilidae
Order	**:**	Liliales
Family	**:**	Smilacaceae
Genus	**:**	*Smilax*
Species	**:**	*china*

MATERIALS AND METHODS:

Plant Collection: Tuberous root of *Smilax china* L. were procured from a local pharmacy, Chennai, Tamil Nadu, India.

Identification and Authentication: Plant material was identified and authenticated by Dr. Sunil Kumar, Research Officer- Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, India. The number is S17100505C. A voucher specimen of the plant was deposited in the herbarium for reference.

Preparation of Powder: Collected tuberous roots were chopped into small pieces and made into powder using a grinding mill. Then the powder was stored in an airtight container.

Chemicals: Formaldehyde, acetic acid, ethanol, phloroglucinol, hydrochloric acid, safranin, glycerine, and all other chemicals used were of analytical grade.

Macroscopical Evaluation: This method is the preliminary evaluation of drugs that can be observed with the naked eye and also with sensory feelings. Shape, size, fracture, texture, surface, external and internal color, odor, and the taste was observed [13-15].

Microscopical Evaluation: Every plant possesses characteristic features, and they can be studied from the cells, tissues, and their arrangements with a suitable staining technique. The collected part is kept in the fixative solution FAA (Formalin 5 ml + Acetic acid 5 ml + 70% ethyl alcohol 90 ml) for more than 48 h. Then the specimens were cut into a thin transverse section and stained with safranin. Photographs were taken with Nikon Eclipse E 200 trinocular microscope attached with a digital camera under bright field light [16-19].

Powder Microscopical Evaluation: A pinch of powder was stained with glycerine and mounted for studying the characters of powdered crude drugs [20-22].

Quantitative Microscopy: Length and width of fibers and stone cells, a diameter of starch grains were measured. These values are unique for specific species. The most important of these values is to identify whether the powdered parts of the plant are adulterated or substituted with inferior quality [23].

Physicochemical Analysis: Physicochemical analysis was performed as per the standard procedures of WHO guidelines, Indian Pharmacopoeia, and Kokate. Ash value like total ash, acid insoluble ash, water-soluble ash and sulphated ash, extractive values like water-soluble and alcohol soluble, loss on drying at 105 ºC and foaming index was performed [24-29].

Fluorescence Analysis: Fluorescence analysis of powdered drug with suitable reagents was performed as per chase method [30, 31].

Determination of Heavy Metal Contamination: Limit tests for heavy metals like arsenic and lead were performed as per the standard procedure present in Indian Pharmacopeia [26].

Test for Arsenic: This test contains the apparatus of 100 ml conical flask closed with a glass stopper, in which a glass tube is passed. The glass tube is placed in a position of at least 3 mm below the lower surface of the stopper. The second tube is placed in such a way that it has to be in contact with the first tube. 50-60 mg of lead acetate cotton is loosely packed, and a small square of mercuric chloride paper is placed to cover the orifice of the tube.

To the conical flask, 1 ml of test solution, 5 m1 of 1 M potassium iodide and 10 gm of zinc AST was added close the container. Repeat the procedure for standard 1 ml of arsenic diluted to 50 ml with

water. After 40 min, any stain produced on the mercuric chloride paper of the sample is compared with the standard.

Test for Lead:

Standard Solution: 1 ml of standard lead solution is taken in a 50 ml Nessler cylinder and dilute with water to 25 ml; adjust the pH between 3 and 4 with dilute ammonia or dilute acetic acid solution. Then dilute to 35 ml with water and mix.

Test Solution: Dissolve the root extract with water to 25 ml and transferred to a 50 ml nessler cylinder. Adjust the pH between 3 and 4 with dilute ammonia or dilute acetic acid solution. Then dilute to 35 ml with water and mix.

Procedure: Add 10 ml of freshly prepared hydrogen sulphide solution to both standard and sample. Mix and dilute to 50 ml with water.

Allow to stand for five min and observe the color produced in both the test and standard. The color produced in the test solution is not more than the color produced in the standard solution.

Qualitative and Quantitative Estimation of Inorganic Elements: The presence of inorganic elements like nitrate, iron, sodium, potassium, magnesium, calcium, sulphate, phosphate, chloride, and carbonate were qualitatively evaluated as per the standard procedure present in Indian Pharmacopeia [21]. Iron content was quantitatively determined.

Quantitative Estimation of Iron: [32]

Preparation of Standard: To 50 mg of the ferrous fumarate, 20 ml of 10% sulphuric acid is added and heated on a water bath for 30 min. Shake well and cool. Then dilute to 100 ml with water. Take 2.5 ml of the resulting solution and dilute to 50 ml with water**.**

Preparation of Sample: The sample solution is prepared similarly to the standard solution with 50 mg of the polyherbal formulation sample.

Procedure: To every 5 ml of standard and sample solutions, add 5 ml of acetate buffer solution of pH 4.7 and 2.5 ml of ascorbic acid solution.

It is kept aside for 15 min at room temperature. To this solution, 2.5 ml of 3% w/v bipyridyl solution is added. Shake well and adjust it to 25 ml with water. Absorbances are measured for both the standard and sample at 523 nm against reagent blank and calculate the results.

Determination of Microbial Contamination: Total viable aerobic count for bacteria and fungi was determined by plate count method [26].

Preliminary Phytochemical Screening: Preliminary phytochemical screening was carried out as per standard procedure to find out the active constituents present in this plant [33-38].

RESULTS:

Macroscopy: *Smilax china* L. is a climbing plant species that come under the genus *Smilax* belonging to the family Liliaceae. Tuberous roots are rough, cylindrical, curved, slightly tapering with brownish or blackish scars. They are about 6 to 12 cm long and 2 to 4 cm wide. Fracture hard, externally brownish-yellow in color, and internally brown in color. No characteristic of odor and taste is slightly bitter in **Fig. 1**.

FIG. 1: MACROSCOPY OF *SMILAX CHINA* L.

Microscopy: Transverse section of the tuberous root is wavy and shows cortex. Cortex is made up of thin-walled, polygonal, elongated mucilaginous parenchymatous cells. Cortex is divided into the outer, middle, and inner cortex. The outer cortex contains elongated parenchyma cells with mucilage, while the inner cortex is made up of polygonal non-mucilaginous cells. The inner cortical cells contain starch grains of varying sizes as the simple and compound type and possess centralized hilum. The inner cortical region contains scattered vascular bundles and is composed of normal elements. Fibers are long and aseptate. A few cells contain raphide crystals of calcium oxalate **Fig. 2.1, 2.2, 2.3**. Scl- Sclereids, Oct-Outer cortex, ICt- Inner cortex, SG- Starch grain, VB- Vascular bundle

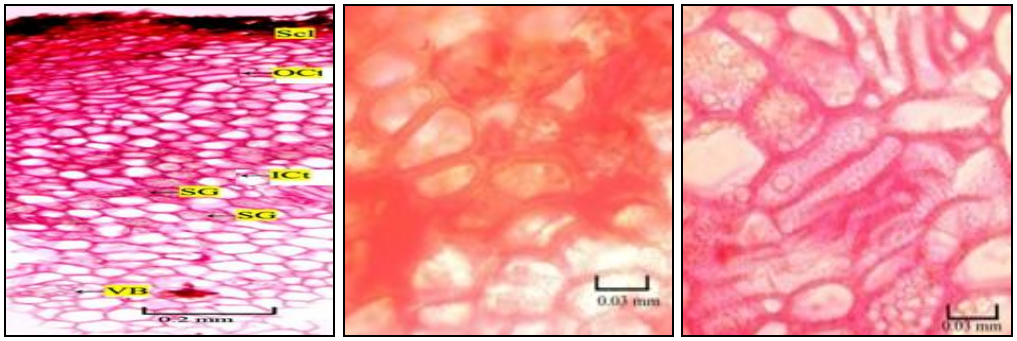

FIG. 2.1: T. S OF *S. CHINA* TUBEROUS ROOT FIG. 2.2 SCLERENCHYMA FIG. 2.3 CORTEX

Powder Microscopy: Powdered drug showed fragments of parenchymatous cells of the cortex, fibers, tannin cells, and vessels with reticulate thickening, sclereids, and various sizes of both simple and compound starch grains **Fig. 3.1, 3.2, 3.3, 3.4**.

Quantitative Microscopy: The results of the length and width of the fibers, stone cells, and diameter of the starch grains were presented in the following **Table 1** and **2**.

Physicochemical Analysis: The results of ash values, extractive values, loss on drying, and foaming index were presented in the following **Tables 3** and **4**.

Fluorescence Analysis: The Fluorescence analysis of *Smilax china* was presented in the following **Table 5**. With ferric chloride, green fluorescence was observed in UV light at 254 nm.

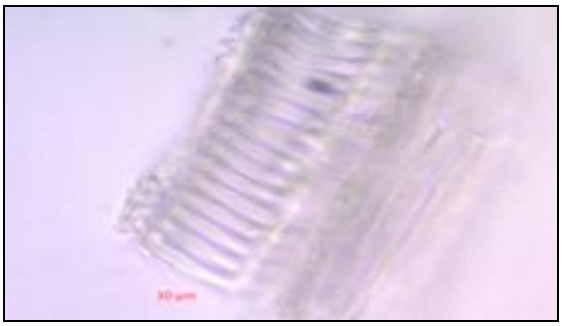

FIG. 3.1: VESSELS

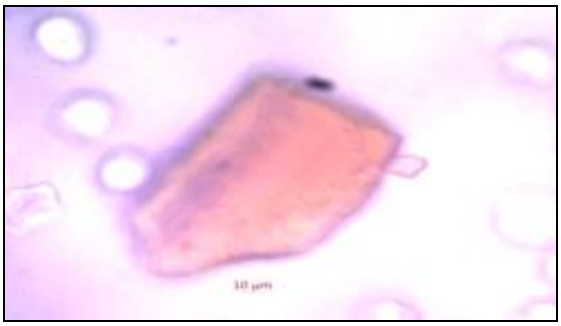

FIG. 3.2: TANNINS

FIG. 3.3: SCLEREIDAL FIBRE

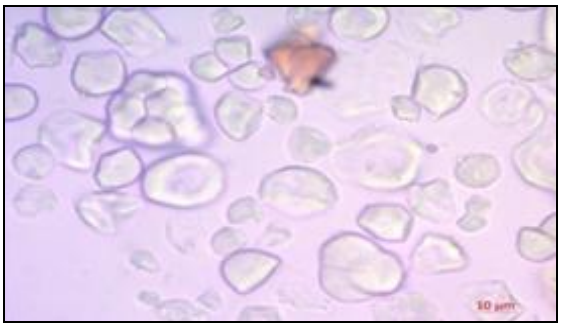

FIG. 3.4: STARCH

TABLE 1: DETERMINATION OF LINEAR MEASUREMENTS OF *SMILAX CHINA*

S. no	Parameters	Length (µm)			Width (µm)		
		Max	Avg	Min	Max	Avg	Min
1	Fibres	234	104	65	91	52	39
2	Stone cells	78	65	52	65	39	26

TABLE 2: DETERMINATION OF DIAMETER OF STARCH GRAINS

S. no.	Name of the Drug	Diameter (µm)		
		Max	Avg	Min
1	*Smilax china*	36	24	24

TABLE 3: DETERMINATION OF ASH VALUES AND EXTRACTIVE VALUES

S. no.	Name of the Drug	Ash Values (% W/W)				Extractive Values (% W/W)	
		Total Ash	Acid insoluble Ash	Water Soluble Ash	Sulphated Ash	Water Soluble	Alcohol Soluble
1	*Smilax china*	0.5	0.06	0.01	0.08	16	6

TABLE 4: DETERMINATION OF LOSS ON DRYING AND FOAMING INDEX

S. no.	Name of the Drug	Loss on Drying (% W/W)	Foaming Index
1	*Smilax china*	4.76	Less than 100

TABLE 5: FLUORESCENCE ANALYSIS OF TUBEROUS ROOT OF *SMILAX CHINA*

S. no.	Treatment	Day Light	Short UV (254 nm)	Long UV (365 nm)
1	Powder	Pale green	Green	Brown
2	Powder + water	Light Brown	Light Green	Light black
3	Powder+1NAlc.NaOH	Yellowish Brown	Light Green	Brownish black
4	Powder +1NAlc. KOH	Pale Brown	Light brown	Yellowish-brown
5	Powder + 1N H_2SO_4	Brown	Brownish black	Black
6	Powder + 1N HCl	Dull white	Pale Green	Brown
7	Powder + 1N HNO_3	Yellowish Brown	Greenish Black	Light green
8	Powder + 1N NaOH	Light Brown	Light Green	Brownish black
9	Powder + 1N KOH	Pale brown	Greenish brown	Reddish-brown
10	Powder + Acetic acid	Dull white	Pale Green	Pale brown
11	Powder + Ammonia	Dull white	Yellowish green	Brown
12	Powder + Ethanol	Light Green	Green	Brown
13	Powder + $FeCl_3$	Yellowish green	Fluorescence green	Dark green
14	Powder + Iodine	Bluish black	Bluish-green	Bluish black

Determination of Heavy Metal Contamination: Limit tests for arsenic showed that the strain produced in the sample is less than that of the standard, and limit tests for lead showed that the color produced in the sample is less than that of the standard.

This showed that the tuberous root of *S. china* is less than that of given standard ppm and free from heavy metal contamination.

Qualitative and Quantitative Estimation of Inorganic Elements: The qualitative estimation of inorganic elements showed the presence of nitrate and iron.

Quantitative Estimation of Iron: Quantitative estimation of iron in the tuberous root of *S. china* was found to be 4.44% w/w.

Determination of Microbial Contamination: Determination of total viable count for bacteria and fungi showed that there was no bacterial and fungal growth.

Preliminary Phytochemical Screening: Preliminary phytochemical screening showed the presence of active constituents like alkaloids, carbohydrates, reducing sugars, phenolic compounds, flavonoids, and terpenoids.

DISCUSSION: Standardization of herbal medicine plays an important role for proper identification, to understand the structure, to gain information about the active constituents, and to know its clinical efficacy. The analysis of macroscopical, microscopical and powder microscopical characters, including physical and chemical parameters, are the confirmatory tests for standardization. So, it is essential to study the pharmacognostic characters of a medicinal plant. The tuberous root of *Smilax china* has medicinal value with clinical applications. However, this plant is not yet having much proven scientific evidence for standardization, even though it has been used by local people to treat various diseases. Pharmacognostical standardization is the basic study and necessary for identification, authentication, and to detect the adulterants. For proper identification of the drug, morphology and anatomical study is the basic and important parameter to study. Morphology of the drug gives touch, sight, color, smell, and taste, which help us to give a primary indication about quality.

Anatomical study shows the presence of cortex, sclerenchyma, elongated parenchyma, starch grains, vascular bundles, fibers, and tannin cells. Linear measurements for fibers, stone cells, and starch grains were measured.

Physicochemical analysis like ash value, extractive value, loss on drying, foaming index and fluorescence study were determined. These are distinguishing characters for determining anatomical structures and setting up the correct identity of this plant *Smilax china* L.

Fluorescence analysis is another important parameter required for basic line standardization, which is unique to the plant and gives the sign of chromophore in the drug. Some plant constituents show fluorescence in the visible light or ultraviolet light when it reacts with suitable reagents because they may be converted into fluorescent derivatives, which are helpful to distinguish them from suspicious specimens as extremely fast techniques. Physicochemical constant evaluation is also another important parameter to identify the adulterants.

Ash value determines the presence or absence of foreign organic matters like metallic salts or silica. Extractive values are useful to get an idea about the phytoconstituents present in the drug. Determination of heavy metal contamination like arsenic and lead revealed that this plant is free from toxic metals like arsenic and lead. So, this drug is safe to use as medicine. Quantitative estimation of iron content showed that this plant contains sufficient iron content, and hence, this plant will also act as a health supplement. This drug is also free from microbial contamination. The preliminary phytochemical screening will divulge the chemical nature of the phytoconstituents present in it. Phytochemical screening showed the presence of alkaloids, carbohydrates, saponins, terpenoids, phenolic compounds, and tannins.

CONCLUSION: From the results of this study, the information obtained may be useful as a reference monograph for *Smilax china* L. for proper identification, authentication, investigation, standardization and helpful to detect the adulterants of the genuine drug. This work is also useful to researchers for further development of studies.

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CONFLICTS OF INTEREST: We declare that we have no conflicts of interest.

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