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Phytopharmacognostical investigation of *Securinega leucopyrus* (Willd.) Muell: An extrapharmacopoeial drug

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Abstract
Securinega leucopyrus (Willd.) Muell, is a thorny large shrub belonging to the family Euphorbiaceae. In Ayurveda it has a lot of potential medicinal uses. It has been described by the medico-ethnobatanist as a wonderful drug for the treatment of wound healing. The aim of present study was to establish standardization parameters of male and female plants of *Securinega leucopyrus* since less reports are available. Pharmacognostical evaluation including examination of morphological, microscopical characters and chemomicroscopy were performed on leaves, stem and roots of both plants. Physicochemical parameters, qualitative and quantitative estimation were performed in leaves sample of both plants because only leaves have proved clinical efficacy. The study reveals the presence of rosette and prismatic crystals of calcium oxalate in leaves of both plants sample. High numbers of prismatic crystals were observed in female plants which suggest its wound healing efficacy. Phytochemical screening of the leaves sampe showed the presence of phytoconstituents like carbohydrate, saponin, protein, flavanoid, glycoside, alkaloid, hexose, reducing sugar, tannin and steroids. These studies would help to establish quality standards, purity and sample identification of *S. leucopyrus.*

Keywords: *katupila*, *Securinega leucopyrus*, chemomicroscopy, phytochemical

Introduction
Securinega leucopyrus (Willd.) Muell, belongs to the family Euphorbiaceae and commonly known in Sri Lanka by "*Katupila*" and in Gujarat by "*Humari*". The tree during fruiting shines with snow white pearly soft fruits covering the tree hence, it is known as Indian snow berry. It is known as bush weed and is an erect shrub 1.5-4-meter-tall with branches cylindrical or obtusely angular when young, grey [1, 2], The plant grows in south eastern Queensland, India, Sri Lanka and Burma [3]. It is known commonly within the Sri Lankan native and folklore practice has long been used as a powerful, important and as a potent medicine internally as well as externally for its healing properties [4]. The plant has been used in preparations of traditional medicines for the treatment of cough, hay asthma, bowel complaints, disinfections, laxatives, diarrhoea, gonorrhoea, constipation, mental illness and kidney stones [5, 6]. Some *in-vitro* studies showed the antimicrobial activity [7]. In Chattisgarh, the decoction of leaves is used to dress the cancerous wounds and is also used in combination with tobacco [8]. A paste prepared by mixing leaves of the plant with tobacco leaves has been used to destroy worms in sores [9]. Decoction of leaves is useful in the treatment of piles and to wash the wounds of cattle. It is also used as popular veterinary medicine [7].
It has been described by the medico-ethno botanist as a wonderful drug for the treatment of wound. It consists of Quercetin, albumin, resins and coloring agents. Leaves of *S. leucopyrus* act as an antiseptic and its paste is used in folklore to extract any extraneous materials from body tissues without surgery [7]. Till date, no phytopharmacognostical work is reported on male and female plants separately. Hence, in this research paper an attempt has been made to establish the genuinity of plant and develops the standard protocol for authentication and standardization of the correct plant material.

Material and methods
Collection of plant material
The fresh leaves, root and stem of male and female *S. leucopyrus* were collected from the surrounding area of Jamnagar in month of August 2016 (22º21'37.656" N70º11'1.056" E). Collected parts were identified by Botanist and compared with characteristics as described in various Floras.[10] Authentication of drug samples (Specimen no. IPGTRA/6187) was done in Pharmacognosy laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar and at Botanical Survey of India, Central National Herbarium, Howrah, West Bengal (CNH/tech.II/2018/80).

Macroscopy
Macroscopical characters of male and female plants of *S. leucopyrus* were studied systematically as per the methods described in the textbooks of Pharmacognosy. The specimens were observed as such with naked eyes and with the help of dissecting microscope. Organoleptic characters of both samples were also observed like color, odour, taste, texture, fracture etc [11].

Microscopy
Free hand sections of leaf, stem and roots were taken from the preserved and fresh materials of both plant samples and observed as such under the microscope. The sections were cleared with chloral hydrate to observe the various cellular contents like crystals of prismatic, rosette, calcium oxalate, calcium carbonate, and silica if, present any. The natures of these crystals were also confirmed by performing some tests like, solubility of them in acids (HCl). The sections were stained with Phloroglucinol and HCl for detecting lignified elements like fibers, sclereids, xylem, vessels, tracheids etc.

Chemomicroscopy
Sections and powders of leaves, root and stem of *S. leucopyrus* were mounted on slides and subjected to treat with various reagents like conc. HCl and acetic acid for confirmation of type of crystals, HCl+ phloroglucinol for lignified content, $FeCl_3$ solution for tannin pigments and iodine for starch grains etc [12, 13].

Phytochemical screening and quantitative estimation
Physicochemical parameters were carried out in both male and female *S. leucopyrus* by using various parameters as mentioned in Ayurvedic Pharmacopeia of India, 2001 [14]. Qualitative chemical tests were carried out in aqueous and methanolic fractions of *S. leucopyrus* powder samples for identifying various phytoconstituents present in the plant species like carbohydrate, saponin, protein, flavanoid, glycoside, alkaloid, hexose, reducing sugar, tannin and steroids [15, 11]. Quantitative analysis was carried out in aqueous extract, for this 1 mg/ml stock solution was prepared and phytoconstituents like carbohydrate [16], protein [17], flavonoid [18], tannin [19], glycoside [20], phenol [21], and terpenoid [22] were quantified using specified standards.

Results
Morphological study
In male plant leaves are simple, petiolated, alternate, elliptic to obovate, entire, blunt somewhat emerginate, 2.5-3.5 cm x 1-1.5 cm in size, upper dark green while, beneath pale, glossy, smooth, glabrous, crowded at the end of branches. In female plant roundish to oval shape leaves were observed. Stem of male plant is cylindrical, erect, branched, mature woody, glabrous, smooth and green in colour while in female plant stem is erect and branches terete or obtusely angular, when young. They are grey in colour and prominently lenticellate; ultimate branchlets spine-tipped, terete and rigid. Tap root, branched, with wiry thin rootlets, straight, smooth, with light longitudinal striations, dark brown in colour are the morphological characteristics of male plant while in female plant, root is of dark brown external while creamish white internally. All other characteristics are same in both plants. [Figure 1]

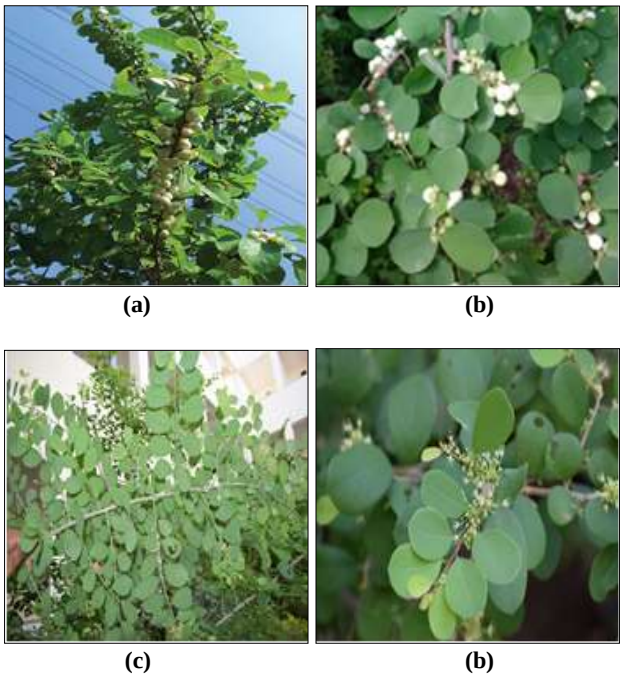

Fig 1: (a) Whole female plant of *Securinega leucopyrus*; (b) Closed view of female plant; (c) Whole male plant of *Securinega leucopyrus*; (d) Closed view of male plant

Microscopical study
The present study revealed the microscopic characters of both male and female leaves of *S. leucopyrus* consists of upper and lower epidermis covered with thick cuticle followed by cortex. Cortex made up of parenchyma cells, number of rosette and prismatic crystals of calcium oxalate, oil globules also present. Section passing through the midrib shows collenchymatous tissue at upper and lower side. Lower and upper epidermis shows the numerous stomata, stomata mainly of anomocytic type. Epidermal cells, prismatic and rosette crystals of calcium oxalate and large quantities of oil globules distributed all over the surfaces. High numbers of prismatic crystals were observed in female plants in comparison to male plant. [Figure 2]
Transverse section of stem of both male and female plants shows the presence of prismatic & rosette crystal, lignified fibres, pitted & spiral vessel, fibre with lumen and oil globules. [Figure 3] Transverse section of root shows thick walled fibres, cork in surface and transverse view, pitted vessel, fragment of cortical thin walled parenchymas, simple and compound starch grains and rosette crystals of calcium

oxalate in both male and female plant of *S. leucopyrus.* [Figure 4]

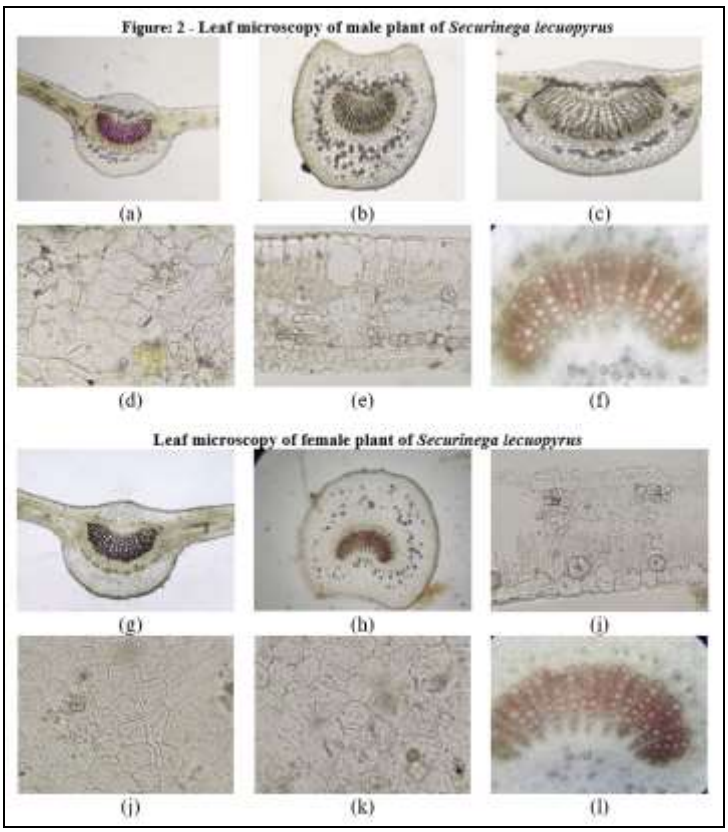

Fig 2: (a) Diagramatic-section; (b) Petiole with prismatic crystal ; (c) Midrib-rosette crystal ; (d) Lower epidermis; (e) Lamina-rosette crystal; (f) Vascular bundle; (g) Diagramatic-section; (h) Petiole-stain with prismatic crystal; (i) Midrib-rosette crystal; (j) Upper epidermis; (k) Lower epidermis; (l) Vascular bundle

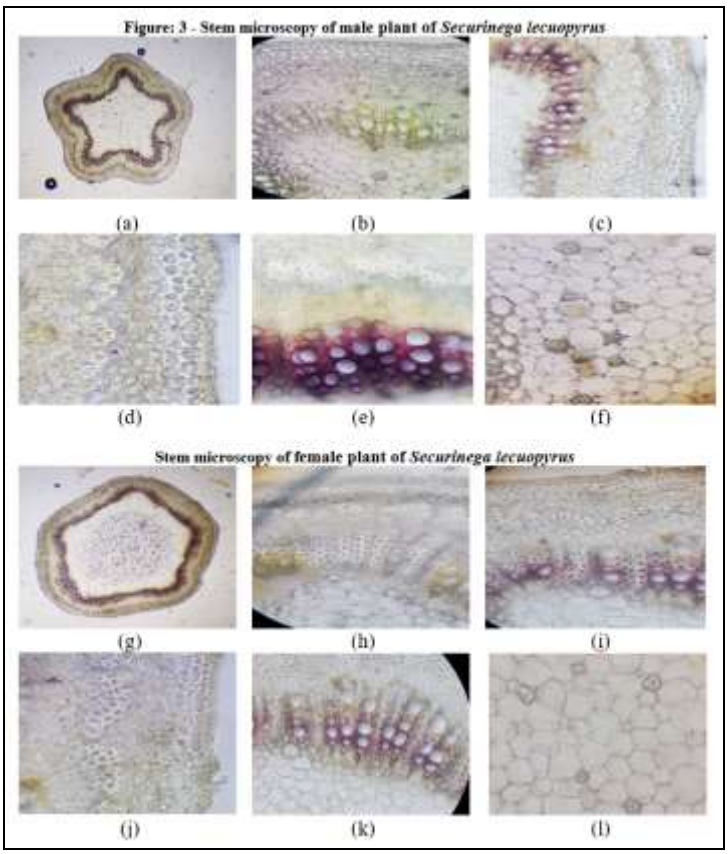

Fig 3: (a) Diagramatic-section; (b) Entire section; (c) Entire stain section; (d) Epidermis, cortex, fibre; (e) Xylem, phloem, pith; (f) Pith; (g) Diagramatic-section; (h) Entire section; (i) Entire stain section; (j) Epidermis, cortex, fibre; (k) Xylem, phloem, pith; (l) Pith

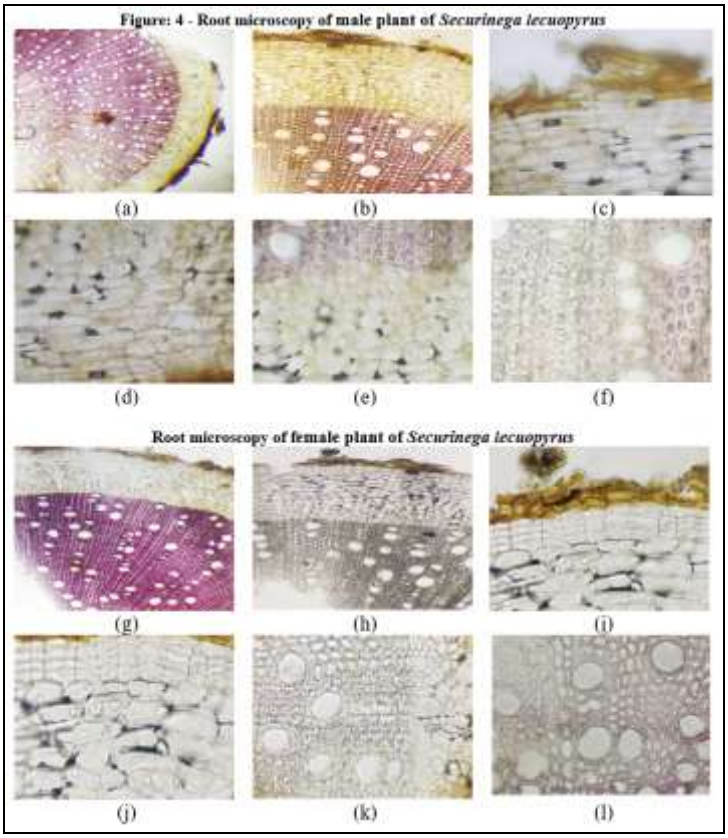

Fig 4: (a) Diagramatic-section; (b) Entire section; (c) Cork in detail; (d) Cortex with starch grain; (e) Phloem; (f) Xylem; (g) Diagramatic-section; (h) Entire section; (i) Cork in detail; (j) Cortex with starch grain; (k) Cortex, phloem, xylem; (l) Xylem, medullary rays

Chemomicroscopy

The chemomicroscopy tests showed lignified cells, calcium oxalate crystals and oil globules in leaves while stem chemomicroscopy of both plants shows the presence of lignified cells, starch, calcium oxalate crystals and oil globules. Calcium oxalate crystals and oil globules were found to be present in roots of both plants. [Table 1]

Table 1: Histo-chemical tests for leaf, stem and root of *S. leucopyrus* male and female plant

Reagents	Observation	Characteristics	Leaf		Stem		Root	
			M	F	M	F	M	F
Phloroglucinol+ Conc. HCl	Red	Lignified cells	++	++	++	++	--	--
	Dissolved	Ca- oxalate crystals	++	++	++	++	++	++
Iodine	Blue	Starch	--	--	++	++	--	--
Fecl3 solution	Dark blue to black	Tannin cells	--	--	++	++	--	--
Sudan III	Red	Oil globules	++	++	++	++	++	++

M: male; F: female, ++: Present, --: Absent

Organoleptic characters

The colour, touch, taste and odour of powder samples of leaves of male and female plant of *S. leucopyrus* were noted down. These characters were useful to having primary idea about the quality of different formulations without using chemical tests. [Table 2]

Table 2: Organoleptic characters of leaves powder of *S. leucopyrus*

Characteristic	*S. leucopyrus*- Male	*S. leucopyrus*- Female
Colour	Light green	Parrot green
Odour	Astringent	Astringent
Taste	Astringent followed by mucilaginous	Astringent followed by mucilaginous
Touch	Smooth	Smooth

Physicochemical analysis

The loss on drying and ash value was almost same in both male and female leaves powder samples. Both samples shows higher water extractive value than methanol extractive value. pH of both samples ranges from 4.6 to 4.9 in 5% and 10% aqueous solutions which was slight acidic in nature. [Table 3]

Table 3: Physicochemical parameters of leaves powder of *S. leucopyrus*

Physicochemical parameters	*S. leucopyrus*- Male	*S. leucopyrus*- Female
Foreign matter (w/w)	--	--
Loss on Drying at 110^0C (% c)	10.98±0.73	11.517± 0.611
Ash value (%w/w)	5.123±0.42	5.267± 0.255

Acid insoluble ash (% w/w)	1.117±0.12	1.313± 0.191
Water extractive value (% w/w)	26.08±1.15	28.430± 1.058
Methanol extractive value (% w/w)	17.23±1.52	18.943± 1.046
pH of 5% w/v aqueous solution	4.60±0.205	4.7± 0.115
pH of 10% w/v aqueous solution	4.70±0.043	4.9± 0.058

Data: Mean±SD (n=3)

Qualitative and quantitative analysis

The qualitative phytochemical test revealed the presence of saponin, glycoside, alkaloid and tannin in methanolic extract while carbohydrate, saponin, glycoside, alkaloid, reducing sugar, tannin and steroid in water extract of both male and female leaves powder of *S. leucopyrus*. [Table 4] The aqueous extract contains highest quantity of carbohydrate followed by, protein, flavonoid, tannin, glycoside, phenol and terpenoid in both plant samples. [Table 5]

Table 4: Qualitative analysis of leaves powder *S. leucopyrus*

Test	Extract of male plant		Extract of female plant	
	Methanol	Water	Methanol	Water
Carbohydrate	-	+	-	+
Saponin	+	+	+	+
Protein	-	-	-	-
Flavanol	-	-	-	-
Glycoside	+	+	+	+
Alkaloid	+	+	+	+
Hexose	-	-	-	-
Reducing Sugar	-	+	-	+
Tannin	+	+	+	+
Steroids	-	+	-	+

++: Present, --: Absent

Table 5: Quantitative estimations of aqueous extract of *S. leucopyrus* leaves

Parameters	*S. leucopyrus*- Male (µg/mL)	*S. leucopyrus*- Female (µg/mL)
Carbohydrate	988.23±28.67	1024.530 ± 39.418
Protein	843.11±11.45	879.11±14.75
Flavanoid	521.48±3.18	531.487 ± 4.648
Tannin	285.34±12.18	302.343 ± 15.437
Glycoside	79.22±8.52	83.22±2.24
Phenol	36.11±2.42	38.107 ± 0.163
Terpenoid	1.742±0.00	1.968 ± 0.000

Data: Mean±SD (n=3)

Discussion

Macroscopic and microscopic evaluation is one of the simplest and cheapest methods for establishing the correct identification of the source of the materials [23]. Results of macroscopic, microscopic study, histochemical tests (chemomicroscopy) of male and female plant suggest that there were no much difference observed between male and female plants of *S. leucopyrus.*

The analysis and quality control of herbal medicines are moving a step ahead towards an integrative and comprehensive direction, in order to tackle the complex nature of herbal medicines. Standardization is a process of confirmation of the identity and determination of the quality and purity of herbal drugs, which is an essential factor for safety, effectiveness and acceptability of the product [24], Standardization and quality control plays a role in establishment of consistent biological activity, a consistent chemical profile for production and manufacturing of herbal drugs.

Organoleptic characters are subjective, sensory judgments based on the experience of the evaluator. They can involve eyeing, feeling, chewing and testing of products to judge for appearance, colour, integrity, texture and flavours. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a sample. Total ash and acid insoluble ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug [25, 26]. Ash value indicates inorganic matter present within the sample, however acid insoluble ash is a good quality control indicator rather than total ash as it indicates the adulteration due to sand and soil. The Loss on drying is indicative of moisture content and volatile oil content. The moisture content in the sample is low which indicates no spoilage of drug due to microbial growth.

Extractive values are useful to evaluate the chemical constituents of crude drug [26]. The water and alcohol soluble extractive value indicate the percentage of soluble polar and moderatly polar component according to polarity of the respective solvent. Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. It was concluded that the water-soluble extractive values are higher than methanol- soluble extractive values, this indicates presence of more amounts of water-soluble contents in the leaves powder samples. The pH value indicates the relative concentration of hydrogen ion in the solution compared with that of standard solution that represents the relative acidity or alkalinity of solution. The pH of 5% and 10% solutions ranges from 4.6 to 4.9 which mean that it is slightly acidic in nature.

Qualitative tests are very useful for the gross detection of chemical moiety in samples. Qualitative test for screening of various functional groups made shows the presence of saponin, glycoside, alkaloid and tannin in methanolic extract while carbohydrate, saponin, glycoside, alkaloid, reducing sugar, tannin and steroid in water extract of both male and female leaves powder of *S. leucopyrus*. Phytoconstituents were also quantitatively estimated in the plant drug. The aqueous extract contains highest quantity of in order of carbohydrate followed by, protein, flavonoid, tannin, glycoside, phenol and terpenoid in both plant samples.

Conclusion

Substitute or counterfeit herbal materials are often found in market thus standardisation of the herbal drugs is necessary to ensure the quality of drug. Phramcognostical profile, chemomicroscopy, physicochemical, qualitative and quantitative parameters can be used as a diagnostic characters of *S. leucopyrus* to facilitate genuinity, quality control, identification, preparation of monograph and to minimize the adulteration. Quantitative estimation of secondary metabolites can be used as a potential source of natural bioactive chemicals and can be provide a great help

for the screening of plant for various activities. These secondary plant metabolites are known to possess various pharmacological effects.

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