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Research

Exploration of Pharmacognostical, Preliminary Phytochemical Investigation, Taxometric Analysis and DNA Barcoding of *Simarouba Glauca* DC.

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Abstract

Background: *Simarouba glauca*, commonly known as Lakmitaru or paradise tree, belongs to Simaroubaceae. It is traditionally referred to as "Dysentery Bark" due to its well-known use against dysentery. The plant is native to Southern Florida, West indies and Brazil. In India, Simarouba *glauca* is traditionally used for the treatment of Helminthic infection, dysentery, hypotension, cancer and diabetes.

Material and methods: Phytochemical investigations revealed the presence of alkaloid, flavonoid, terpenoid, anthraquinone, steroids, phenol, saponin, tannin, carbohydrate. The plant has demonstrated various pharmacological activities, including antibacterial, antifungal, antioxidant, anticancer, anti-proliferation and pro-apoptotic activity, anti- inflammation, antimicrobial, antimalarial, antiamoebic, antiulcer, hepatoprotective, reducing patchy skin saturation, haemolytic, acaricidal, hypertensive, antidysentery, analgesic and anti-leukemic activities. In the present study, fresh leaves of *Simarouba glauca* were collected, authenticated, shade dried, coarsely powdered and extracted using Aqueous solvent system. The extract was concentrated and stored in air tight container for further analysis.

Result and discussion: Macroscopical and microscopical examinations were carried out for the identification of the plant in both whole and powdered forms, while supplementary analyses including powder microscopy, physicochemical evaluation, fluorescence analysis, taxometric studies and DNA barcoding further confirmed its authenticity.

Conclusion: The present article draws phytochemical analysis of the plant shows presence of beta carboline alkaloid, flavonoid, tannin, carbohydrate, steroid, terpenoid and quantitatively estimates harmaline(0.0079mg/g), quercetin (0.0055mg/g), gallic acid(0.0009mg/g), tannic acid (0.0018 mg/g) and thymol (0.0070mg/g) equivalent in *Simarouba glauca*.

Keywords: Aqueous extract, DNA barcode, Lakmitaru, Pharmacognostical, Physicochemical, Taxometrics.

INTRODUCTION

Simarouba glauca, commonly known as '**Lakmitaru**' or '**Paradise tree'** belongs to Simaroubaceae. It is indigenous to Southern Florida, West Indies and Brazil [1], is considered exotic in India, Sri Lanka, Philippines and Myanmar [2]. It was first introduced by National Bureau of Plant Genetic Resources in the research station at Amravati in Maharashtra in 1966[3,4,5] and later to the University of Agricultural Sciences, Bangalore in 1986.

Paniyas Tribes of Wayanad district, Kerala used the leaf decoction to treat cancer [6] some communities of Shimoha, Karnataka [7]. In Cuban folk medicine, the leaves are used for helmintic infection, dysentery and to exert hypotensive action [8]. Phytochemical survey reported the presence of alkaloid, flavonoid, terpenoid, anthraquinone, steroids, phenol, saponin, tannin, carbohydrate [9].It exhibited antibacterial [10], antifungal[11], antioxidant[12], anticancer[13], anti-proliferation and pro-apoptotic avtivity[14], anti-inflammation[15], antimicrobial[16], antimalarial[17], antiamoebic[18], antiulcer[19], hepatoprotective[20], reducing patchy skin saturation[21], haemolytic[22], acaricidal[23], hypertensive[24], antidysenteric[25], analgesic[26], anti-leukemic activitiy[27-28]. It is insistent to explore the pharmacognostical parameters of the leaf and the present investigation addressess the macroscopic and microscopical evaluation, determination of physico-chemical constants of *Simarouba glauca* leaves, along with Taxometric studies and DNA barcoding.

MATERIALS AND METHODS

Collection and Authentication

Leaves ware collected from the village of Nazereth, Thoothukudi, Tamil Nadu in the month of February 2025. The leaves were identified and authenticated by Dr. S. Mutheeswaran, Scientist, Xavier Research Foundation, St. Xavier's College, Palayamkottai, Tamil Nadu. The herbarium of this specimen was kept in the department for further reference.

Pharmacognostical evaluation

Fresh leaves of *Simarouba glauca* (L) were subjected to Pharmacognostical studies, including organoleptic evaluation, macroscopic and microscopic examination.

Organoleptic evaluation

Fresh leaves of *Simarouba glauca* were collected and evaluated for organoleptic characteristics such as colour, odour and taste using sensory perception and results are in table 1.

Macroscopical evaluation

macroscopic characteristics of the leaves were studied, parameters such as length, width, leaf base, apex, phyllotaxy and venation pattern. The observations were documented and presented in Figure: 1 & 2 and Table: 1.

Microscopical evaluation

The leaves were preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% Safranin and 0.5% Astra blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 colour digital camera under bright field light. Magnifications were indicated by scale bar [29]. The results were presented in the corresponding figures 3 & 4.

Histochemical studies

Sections of the leaves of *Simarouba glauca* were stained using specific reagents such as N/50 iodine, phloroglucinol with conc. HCl, picric acid and Potassium hydroxide to detect and localize starch, lignin, alkaloids and tannin respectively. The staining protocols and corresponding observations are presented in Figure 5.1 to 5.4 and Table :2.

Determination of leaf constants

Leaf fragments of about 5 x 5 mm in size were placed in a test-tube containing about 5 ml of saturated aqueous solution of chloral hydrate and heated in water bath for 10-15 minutes. Then it was kept on slide and mount it in glycerin. Examine under a microscope with a 4x objective and a 10x eyepiece, equipped with a camera lucida. Leaf parameters such as epidermal number, Stomatal number, Stomatal index, Vein islet number, Vein termination number and Palisade ratio are determined [30]. The results were summarized in Table no:3

Preparation of powder

Collected leaves were washed thoroughly, shade-dried and coarsely powdered. The dried powder was then passed through sieve No. 40 to obtain a uniform particle size for further analysis.

Powder microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence

of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented [31]. The results were summarized in Figure 6.

Behaviour of powder with various chemical reagents

A small quantity of the powder was treated with few drops of various freshly prepared chemical reagents. The resulting colour changes were observed under visible light, UV short and long wavelength [32]. The observations were presented in Table no: 4.

Physicochemical analysis

The powdered leaves were subjected to various physicochemical analyses, including determination of foreign matter, loss on drying, total solids, ash values and extractive values using different solvents. These parameters were evaluated in accordance with the procedures prescribed in the Ayurvedic pharmacopoeia [33], and the results are presented in Table no:5. The ash obtained was further analysed for the presence of inorganic elements [34].

Preparation of fresh juice of *Simarouba glauca*

Fresh leaves of 25g were weighed and cut into small pieces then add 30 ml water and made into a fresh juice of 30 ml.

Preliminary Phytochemical screening

Preliminary phytochemical screening was carried out using standard procedures and specific reagents to identify the presence of various phytoconstituents [35]. The results of the screening are summarized in Table no:6.

Determination of LOD and LOQ

Limit of Detection (LOD)

LOD for an individual analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantified.

Limit of Quantification (LOQ)

LOQ for an individual analytical procedure is the lowest amount of an analyte in a sample that can be both detected and quantified.

Procedure

Fresh juice of *Simarouba glauca* was prepared and scanned across the UV range of 200–700 nm to identify the presence of peaks, and the corresponding wavelengths were recorded. Subsequently, juice samples at concentrations ranging from 0 to 10 µg/ml were prepared and analyzed using UV spectroscopy at the previously identified wavelength. The LOD and LOQ values were calculated based on the standard deviation of the response and the slope obtained from the calibration curve of *Simarouba glauca* juice [36,37]. The obtained results were displayed in table 7 and figure 7.

Quantitative estimation of Phytoconstituents

- Determination of Gallic Acid Equivalent
- Determination of Tannic acid Equivalent
- Determination of Quercetin Equivalent
- Determination of Thymol Equivalent
- Determination of Harmaline Equivalent

Determination of Gallic Acid Equivalent of *Simarouba glauca* leaf

A series of calibrated 10ml volumetric flask is taken and standard solution (gallic acid) and aqueous juice of *Simarouba glauca* solution of various concentrations (5µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) is taken. To each of this solution add 5ml of distilled water and 0.5ml of 1N Folin's Cio-calteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made up to 10 ml with distilled water. It is allowed to incubate for 2 hours at room temperature. Intense blue colour is developed. The reaction mixture without sample is used as blank. After incubation, absorbance is measured at 725nm using UV spectrophotometer and the mean values will be recorded. The calibration curve will be plotted using standard gallic acid. Total phenolic content of *Simarouba glauca* leaf juice is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) [38]. The results are illustrated in Figure 7 and summarized in Table 7.

Determination of Tannic acid Equivalent of *Simarouba glauca* leaf

Prepare various concentration aqueous juice of *Simarouba glauca* into test tubes. To this, 0.5ml of Folin-Denis reagent and 0.8mL of distilled water was added. The tubes were kept aside for 15min. To this, 1mL of 10% sodium carbonate solution was added and the remaining volume was made up with 7.5mL of distilled water. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min. Tannic acid, used as a standard was taken at different concentration 5,10,15,20 mcg/ml in different test tubes and the procedure adopted above

was followed. The calibration curve for tannic acid was plotted using concentration versus absorbance. A linear regression equation was calculated and the equation was used to calculate the amount of total tannins as tannic acid equivalent. Total Tannin content of *Simarouba glauca* leaf juice is expressed in terms of mg of Tannic acid equivalent per gm of extract (mg TAE/g) [39]. The results are illustrated in Figure 8 and summarized in Table 8.

Determination of Quercetin Equivalent of *Simarouba glauca* leaf

Total flavonoid content was measured with the aluminium chloride colorimetric assay. A series of calibrated 10ml volumetric flask were taken and standard solution (Quercetin) and aqueous juice of *Simarouba glauca* solution of various concentrations (10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml) were taken. To each of these solutions add 4ml of water and 0.3ml of 5% sodium nitrite solution is added. After 5 minutes, 0.3ml of 10% aluminium chloride is added. At 6th minute, 2ml of 1M sodium hydroxide is added. Finally, volume is made up to 10ml with distilled water and mix well. Orange yellowish colour is developed. The absorbance is measured at 510 nm spectrophotometer using UV-visible spectrophotometer and the mean values will be noted. The blank is performed using distilled water. The calibration curve is plotted using standard Quercetin. The total flavonoid content in the juice is expressed as milligrams of Quercetin equivalent per gram of extract (QE/g) [38]. The results are illustrated in Figure 9 and summarized in Table 9.

Determination of Thymol Equivalent of *Simarouba glauca* leaf

Weigh 2g of dried plant leaf powder of *Simarouba glauca* and transfer it to a clean test tube. Add 10 mL of methanol to the leaf powder, shake thoroughly, and allow the mixture to stand. Filter the solution carefully to obtain a clear methanolic extract. Pipette 5mL of the methanolic *Simarouba glauca* into a clean test tube. Add 2mL of Chloroform to the extract and mix well. Add 3mL of concentrated sulfuric acid carefully into the mixture. Allow the reaction to proceed for the development of colour. Measure the absorbance at 538 nm using a UV-Visible spectrophotometer. The calibration curve will be plotted using standard Thymol in the concentration range of 2-10µg/ml. Total Terpenoid content of methanolic extract of *Simarouba glauca* is expressed in terms of mg of Thymol equivalent per gm of extract (TE/g) [40]. The results are illustrated in Figure 10 and summarized in Table 10.

Determination of Harmaline Equivalent of *Simarouba glauca* leaf

Estimation of total alkaloid content, a known weight of the dried extract was dissolved in a minimal amount of 2N HCl, filtered, and the volume was adjusted with distilled water to a suitable concentration (e.g., 1 mg/ml). One millilitre of this acidified extract was transferred to a separatory funnel and washed three times with 10 ml chloroform to remove non-alkaloid components, discarding the chloroform layers each time. The aqueous layer was then neutralized to approximately pH 7 using 0.1N NaOH, followed by the addition of 5 ml of bromocresol green (BCG) solution and 5 ml of phosphate buffer (pH 4.7). This mixture was shaken and extracted with chloroform in increasing volumes (1 ml + 2 ml + 3 ml + 4 ml) to obtain a total of 10 ml, and all chloroform layers were combined and made up to 10 ml in a volumetric flask using chloroform. For the calibration curve, various concentrations of standard Atropine solution (0.4, 0.6, 0.8, 1.0, and 1.2 ml of 100 µg/ml) were processed using the same extraction procedure, and the absorbance of the resulting chloroform extracts was measured at 470 nm against a reagent blank. The total alkaloid content was expressed as mg Harmaline Equivalent per gram (mg HE/g) [41]. The results are illustrated in Figure 11 and summarized in Table 11.

Taxometric analysis

Taxometric analysis was performed based on six morphological characters: leaf blade, venation, apex, base, margin and shape as described in reference [42]. The analysis and results are presented in Figure 12 & 13 and Table 12 & 13.

DNA Barcoding

Genomic DNA Isolation

About 100 mg of plant tissue was ground with liquid nitrogen to make fine powder using mortar and pestle. Added 1 ml of preheated CTAB extraction buffer with 20µl of β-mercapto ethanol to the mortar and finely ground. The contents were transferred into a 2 ml centrifuge tube and incubated for 20 to 30 min at 65°C on a water bath. After centrifuging using Refrigerated Centrifuge (Eppendrof, 5418R) the tube at 12,000 rpm for 10 min the supernatant was transferred to a fresh centrifuge tube and added equal volumes of chloroform: isoamyl alcohol (24:1) mixture and mixed gently by inverting tubes till an emulsion was formed. The tubes were centrifuged at 13,000 rpm for 12 min. The clear aqueous phase was transferred to fresh centrifuge tubes and equal volumes of ice-cold isopropanol was added. The sample was incubated overnight at -20° C and centrifuged at 12000 rpm for 3 min. The supernatant was discarded and the pellet was washed with 70% ethanol. DNA pellets were then air-dried at room temperature by allowing evaporation of uncovered centrifuge tubes. The pellets were suspended in an appropriate volume (20-30 µl) of $T_{10}E_1$ buffer [43].

Identification of DNA

The quality and concentration of genomic DNA were checked by running the DNA sample on 1% agarose gel. The DNA concentrations were rechecked by visual assessment of band intensity under UV-trans-

illuminator (Biorad, GelDoc Go, USA). The quantity of 1 µl of isolated DNA was checked using Nanodrop (Thermoscientific, Nanodrop One, USA).

PCR Amplification

The DNA barcode candidate rbcL was used for PCR amplification as the same resulted in amplification. The isolated DNA was used as a template for PCR reaction and carried out in a thermocycler (Applied Biosystem, Veriti ™, USA). The PCR products were then loaded onto 1% agarose gel and the amplification was confirmed.

Sequence analysis and submission in NCBI

FASTA format of the nucleotides were obtained using Finch TV from the chromatogram. The FASTA was fed into Basic Local Alignment Search Tool (BLAST) algorithm of NCBI to identify the closest matching sequence in the nucleotide database of GenBank. After confirmation of the species, the sequence was submitted to NCBI with the necessary details to obtain GenBank ID. The sequence was converted to Barcode using the software BioRad barcode generator.

Genomic DNA was isolated from the authenticated sample, and its quality was assessed spectrophotometrically, followed by agarose gel electrophoresis was displayed in Table 14, Fig.14. The genomic DNA concentration and absorbance (A_{260}/A_{280}) ratio are depicted in Table 14. The PCR-amplified products after electrophoresis were subjected to gel documentation with a 100 bp DNA ladder. The sequence was obtained using ITS as the marker is displayed in Fig. 15, converted to a barcode in table 15 [44].

RESULTS AND DISCUSSION

Pharmacognostical evaluation

Macroscopical evaluation

The leaves of *Simarouba glauca* are pinnately compound, with lanceolate to oblong leaflets. They exhibit an alternate arrangement, an entire or slightly undulating margin, and a smooth, glossy green upper surface. The leaves are 5–15 cm long and 2–6 cm wide, with a bitter taste and mild odor.

Fig 1: Habitat of *Simarouba glauca*

Fig 2: Dorsal & ventral surface

Table 1: Macroscopic studies of *Simarouba glauca*

S.No	Characters	Observation
1	Colour	Green
2	Odour	Mild or slightly bitter
3	Taste	Very bitter due to presence of quassinoids
4	Type	Pinnately compound
5	Length	5-15 cm
6	Width	2-6 cm
7	Thickness	Moderately thin
8	Leaflet shape	Lanceolate to oblong
9	Leaf arrangement	Alternate
10	Leaf margin	Entire or slightly undulating
11	Leaf apex	Acute to acuminate
12	Leaf base	Cuneate (widge-shaped)
13	Leaf surface	
	• Dorsal surface	Smooth,glossy green
	• Ventral surface	Pale green, slightly pubescent

14	venation	Pinnate (prominent midrib with lateral veins)
15	Texture	Thin to slightly coriaceous (leathery)
16	Petiole	Short and sturdy

Microscopic evaluation

Leaf

Transverse Section of leaf shows slightly elevated upper and convex shaped lower midrib surface with lateral laminar extensions.

Midrib

TS of midrib shows single layered upper and lower epidermis covered by thick cuticle and bears papilla like trichomes on lower side; upper epidermis is formed of tangentially elongated barrel shaped cells while lower epidermis consists of small rounded to oval shaped cells; 3 to 4 layers of collenchymatous hypodermal layer is present below the Epidermis followed by parenchymatous ground tissue embedded with a large nearly concentric vascular bundle; 3 to 4 layer thick pericyclic fibers covers the entire bundle followed by phloem tissue which surrounds the xylem elements; a small emerging bundle can also be seen at the central pith region, along with small secretory canal.

Lamina

TS of lamina shows single layered upper and lower Epidermis covered by thick cuticle; upper epidermis is formed of tangentially elongated barrel shaped cells while lower epidermis consists of small rounded to oval shaped cells and bears papilla like trichomes on lower side; mesophyll tissue is differentiated into upper compactly arranged columnar cells of palisade layer followed by 3 to 4 layers of spongy parenchymatous cells; veins can be seen traversing through the mesophyll tissue; plenty of astrosclereids are found scattered throughout the lamina.

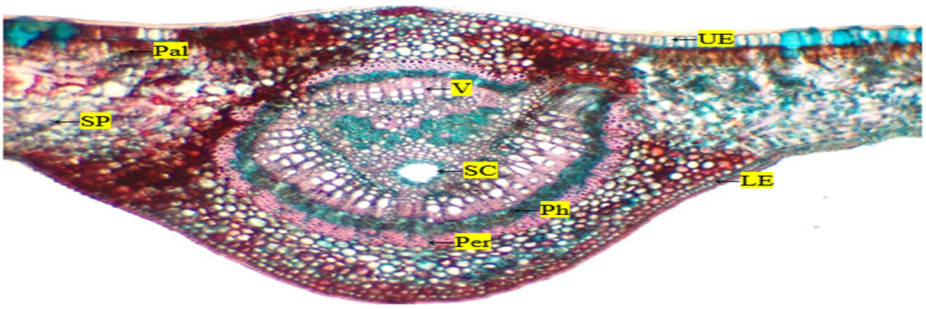


Fig 3: TS of *Simarouba glauca* leaf passing through midrib

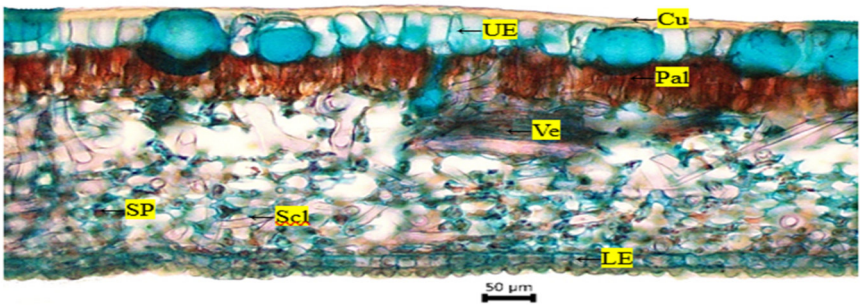


Fig 4: TS of Lamina

Cu - cuticle; **LE** - lower epidermis; **Pal** - palisade cells; **Per** - pericycle; **Ph** - phloem; **SC** - secretory canal; **Scl** - sclereids; **SP** - spongy parenchyma; **UE** - upper epidermis; **V** - vessel; **VB** - vascular bundle; **Ve** - vein

Histochemical analysis

Table 2: Histochemical studies of *Simarouba glauca* leaf

S.no	Reagents	Test	Nature of change	Observation
1	Phloroglucinol+ HCL	Lignin	Pink	vessels
2	Iodine solution followed by sulfuric acid	Starch	Brown	Parenchyma cells
3	Picric acid	Alkaloids	Yellow	Prismatic crystals
4	Heating with KOH	Tannin	Green	palisade cells

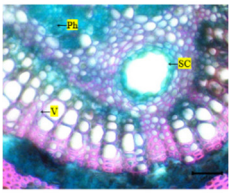


Fig 5.1: V

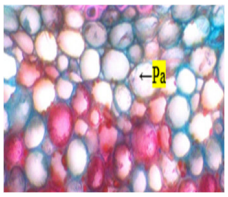


Fig 5.2: Pa

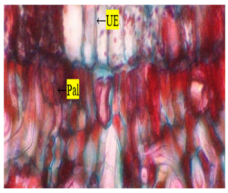


Fig 5.3: Pal

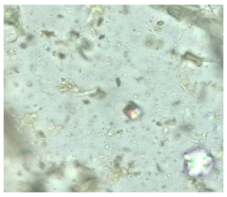


Fig 5.4:Pr

V-vessel; **Pa**; parenchyma cells; **Pal**;palisade cells; **Pr**; Prismatic crystal

Determination of leaf constant

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded and the leaf is hypostomatic with anomocytic stomata on lower epidermis.

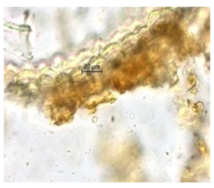
Table 3: Quantitative microscopy of *Simarouba glauca* leaf

Parameters	Upper epidermis (cells/mm^2)	Lower epidermis (cells/mm^2)
Epidermal number	1800 - 2000	1800 - 1900
Stomatal number	-	300 - 350
Stomatal index	-	14.28 - 15.5
Palisade ratio	3 – 4	
Vein islets number	1-2	
Vein termination number	3-4	

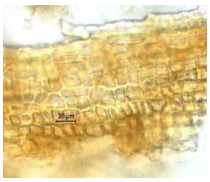
Powder analysis

Powder microscopy

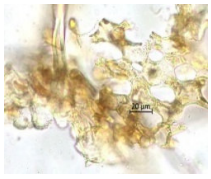
The powder is brownish green coloured with characteristic odour and bitter taste; shows the characters like fragments of epidermis with cuticle, surface view of epidermis, collenchyma cells, fragments of parenchyma cells, fibres, crystal fibre, vessels with spiral and reticulate thickenings, and prismatic crystals.



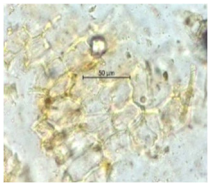
Cuticle



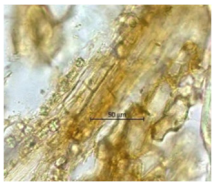
Epidermis



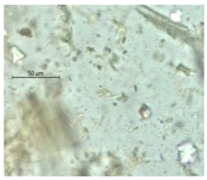
Collenchyma



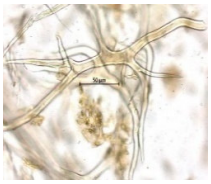
Parenchyma cells



Crystal fiber

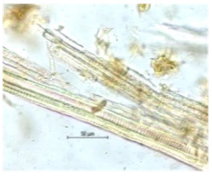


Prismatic crystal

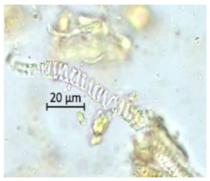


Astrosclereid

Tracheid



Reticulate Vessel



Spiral vessel

Fig 6: Powder microscopy of *S. glauca*

Behavior of powder with various chemical reagents

The powdered leaf of *Simarouba glauca* was treated with various chemical reagents and the results were depicted in Table 4.

Table 4: Behavior of Simarouba glauca powder with various chemical reagents

S.No	Sample And Reagent	Observation		
		Visible	UV (365 nm)	UV (265 nm)
1	Powder	Light green	Light green	Pale green
2	Powder + 1N HCl	Light brownish green	Light green	Green
3	Powder + 1N NaOH	Dark brownish green	Dark green	Dark green
4	Powder + 50% H2SO4	Light green	Bluish green	Dark green
5	Powder + 50% KOH	Brownish green	Green	Dark green
6	Powder + Conc.H2SO4	Dark green	Brown	Blackish green
7	Powder + Conc.HNO3	Brick red	Dark green	Yellowish green
8	Powder + 50% HNO3	Light brownish yellow	Light green	Light green
9	Powder + Conc.HCl	Dark green	Light green	Dark green
10	Powder + Iodine solution	Darkish purple	black	Greenish black
11	Powder + Acetic acid	Green	Brown	Dull green

Physicochemical analysis

Table 5: Determination of Physicochemical parameters of *Simarouba glauca* leaf

S.No	Physiochemical Parameters	Result
1	Foreign matter	Nil
2	Loss on drying	17.67 ±6.12 % w/w
3	Total solid	99.64±0.122 % w/w
4	Ethanol soluble extractive	15.43±6.12 % w/w
5	Ethyl acetate soluble extractive	5.9±0.61% w/w
6	Petrolium ether soluble extractive	2.83±0.98 % w/w
7	Chloroform soluble extractive	1.4±0.43 % w/w
8	Water soluble extractive	4.43±1.40 % w/w
9	Total ash	9.3±2.26 % w/w
10	Acid insoluble ash	8.3±1.03 % w/w
11	Water soluble ash	6.73±2.95 % w/w
12	Inorganic elements	Presence of Chloride and Sulphate
13	Heavy metals (lead)	Nil

The ash values of the plant were estimated using standard procedures which showed a total ash of 8.3±1.03% w/w, water soluble ash 6.73±2.95% w/w and acid insoluble ash of 9.3±2.26% w/w. Loss on drying and total solid value of the powder was determined as 17.67±6.12% w/w & 99.64±0.122% w/w respectively. Ethanol extractive, Ethyl acetate extractive, Petroleum ether extractive, Chloroform extractive and Water extractive, the % yield of the extractive was found to be 15.43±6.12% w/w, 5.9±0.61% w/w, 2.83±0.98% w/w, 1.4±0.43% w/w and 4.43±1.40% w/w respectively. To the ash of *Simarouba glauca*, leaves was treated with 50% v/v hydrochloric acid and kept for 1 hour. It was filtered; filtrate was used for inorganic and heavy metal analysis using various reagents. The quantitative estimation inorganic elements and heavy metal analysis of *Simarouba glauca* leaves reveal the presence of chloride, sulphate.

Preliminary phytochemical screening

Table 6: Determination of the phytochemical analysis *Simarouba glauca leaf*

S.no	Phytochemical Analysis	Observation
1	Test for Carbohydrate	+
2	Test for Flavonoids	+
3	Test for Alkaloids	+
4	Test for beta carboline alkaloid	+
5	Test for Saponins	_

6	Test for Tannins	+
7	Test for Amino acid	_
8	Test for Protein	+
9	Test for Glycoside	_
10	Test for Starch	_
11	Test for terpenoid	(trace)
12	Test for Phenolic compound	+
13	Test for Anthraquinone	+
14	Test for Anthocyanin	+
15	Test for Sterol	+
16	Test for gums & mucilage	+

(+ Present; - Absent)

Phytochemical screening of *Simarouba glauca* leaves reveals the presence of Carbohydrate, Alkaloids, Flavonoid, Anthraquinone, anthocyanin,beta carboline alkaloid, Tannin, Protein, terpenoid, Phenol, sterol, gums & mucilage. This test identifies the bioactive compounds in medicinal plants aides in drug discovery.

LOD and LOQ of *Simarouba glauca*

Various concentrations (0 to 10 µg/ml) of *Simarouba glauca* fresh juice were prepared as blank, whose absorbance was observed under UV (Model: Shimadzu UV-1900i) at 340 nm and displayed in table 7.

Table 7: Determination of LOD and LOQ of *S. glauca* fresh juice

S. No	Concentration (µg/ml)	Absorbance of *S. glauca* fresh juice
1	1	0.0353
2	2	0.0529
3	3	0.228
4	4	0.327
5	5	0.442
LOD		**0.2359**
LOQ		**0.7149**

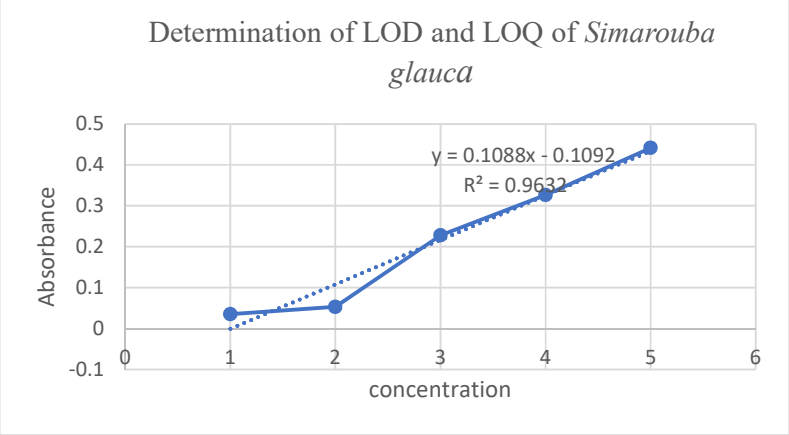


Fig 7: Determination of LOD and LOQ of *Simarouba glauca*

Taxometric analysis

The genus of *Simarouba* comprises approximately 6 accepted species, primarily distributed across Central and South America, as well as parts of the Caribbean. The recognized species include;
The characters such as leaf blade, venation, apex, base, margin and shape are considered.
Leaf blade – Simple, oblanceolate to oblong- lanceolate: present -1, absent-0
Leaf venation – Pinnate: present -1, absent-0
Leaf apex – Acute to acuminate: present -1, absent-0

Leaf base – Cuneate to attenuate: present -1, absent-0
Leaf margin – Entire or slightly undulate: present -1, absent-0
Leaf shape – oblanceolate or elliptic-lanceolate: present -1, absent-0

Table 8: Character Matrix of *Simarouba* Species

Species	Blade	venation	Apex	Base	Margin	Shape
Simarouba glauca	1	1	1	1	1	1
Simarouba amara	1	1	0	1	0	1
Simarouba berteroana	1	0	1	0	1	0
Simarouba laevis	1	1	1	1	1	0
Simarouba tulae	1	0	0	0	1	0
Simarouba versicolor	1	1	0	1	1	1

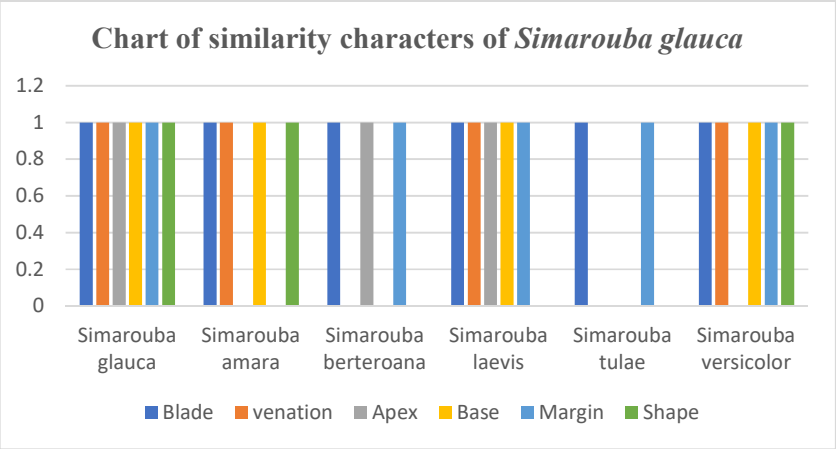


Fig 8: Chart of similarity characters of *Simarouba glauca*

Table 9: Dissimilar Character Matrix of *Simarouba* Species

Species	*S. glauca*	*S. amara*	*S. berteroana*	*S. laevis*	*S. tulae*	*S. versicolor*
Simarouba glauca	0	0.17	0.5	0.17	0.83	0.17
Simarouba amara	0.17	0	0.5	0.17	0.67	0.33
Simarouba berteroana	0.5	0.5	0	0.5	0.67	0.5
Simarouba laevis	0.17	0.17	0.5	0	0.67	0.33
Simarouba tulae	0.83	0.67	0.67	0.67	0	0.83
Simarouba versicolor	0.17	0.33	0.5	0.33	0.83	0

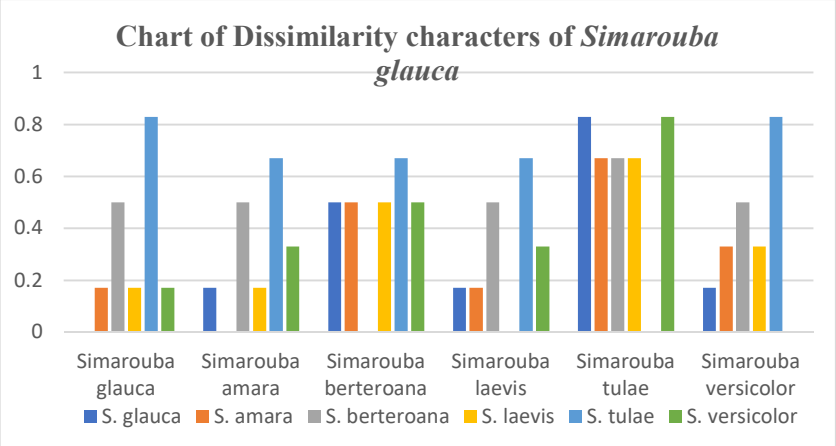


Fig 9: Chart of Dissimilarity characters of *Simarouba glauca*

Sample Matching Coefficient

SSM=NS/NS+ND * 100

SSM values of six species are:

- *Simarouba glauca*-23.3%
- *Simarouba amara*-23.3%
- *Simarouba versicolor*-23.3%
- *Simarouba berteroana*-43.3%
- *Simarouba laevis*-23.3%
- *Simarouba tulae*-56.7%

The sample matching coefficient (SSM) was used to compare *Simarouba glauca* with its related species based on leaf characters. The results showed that *S. glauca* is most similar to *S. tulae* with an SSM value of 56.7 %, indicating they share more common leaf features.

Simarouba berteroana showed a moderate similarity (43.3%) with *S. glauca*, while *S. amara, S. versicolor* and *S. laevis* had only 23.3 % similarity, showing that they are more different from *S. glauca*.

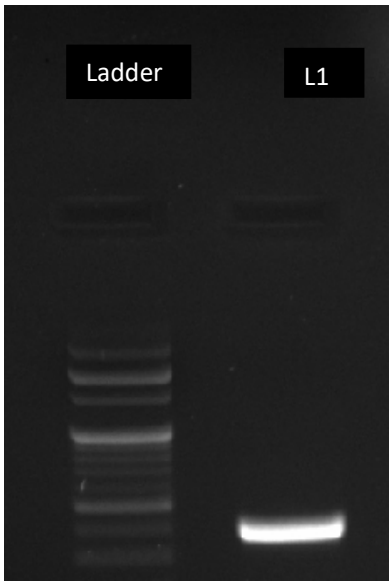
Form these results, it is clear that *S. tulae* is the closest species to *S. glauca*, while *S. amara, S. versicolor* and *S. laevis* are the most distant. This proves that leaf morphology is useful for identifying and differentiating species in the *Simarouba* genus.

DNA barcoding

Genomic DNA was successfully isolated from the authenticated *Simarouba glauca* sample, exhibiting good purity as indicated by spectrophotometric analysis (A260/280 = 1.87 and A230/260 = 2.16). DNA integrity was confirmed through agarose gel electrophoresis, as shown in Table 15 and Figure 15. PCR amplification using ITS primers produced clear, specific amplicons, which were verified via gel electrophoresis and documented using a 100 bp DNA ladder. The ITS-based sequencing yielded high-quality nucleotide data that matched *Simarouba glauca* in BLAST analysis, as presented in table 16. The obtained sequences were converted into DNA barcodes and submitted to GenBank providing reliable molecular identification of the species. These results validate the use of ITS-based DNA barcoding for the authentication and genetic characterization of *Simarouba glauca*.

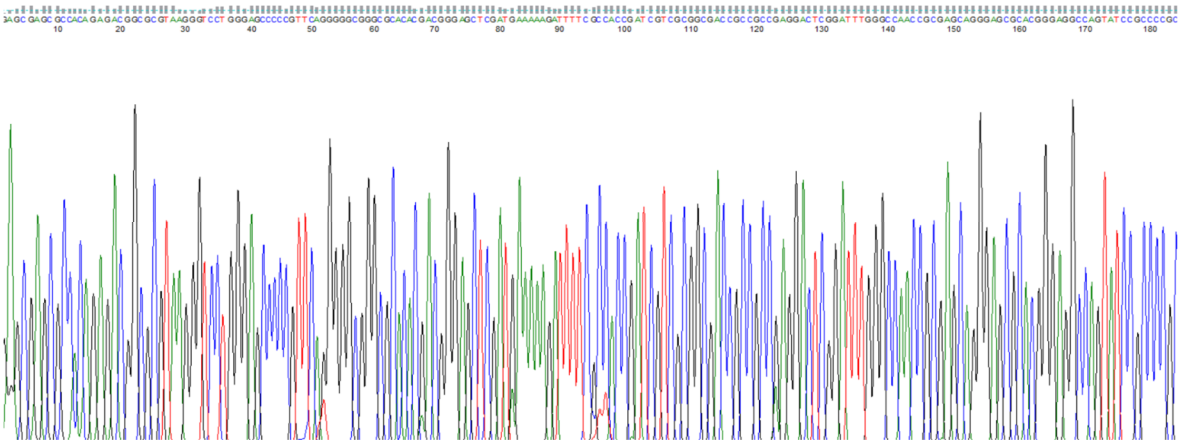
Table 10: Quality check and quantification of DNA

Sample code	Concentration (ng/µl)	$A_{260/280}$	$A_{230/260}$
Simarouba glauca	232.00	1.87	2.16



L1. *Simarouba glauca* genomic DNA

Fig 11: Gel image of PCR amplified product

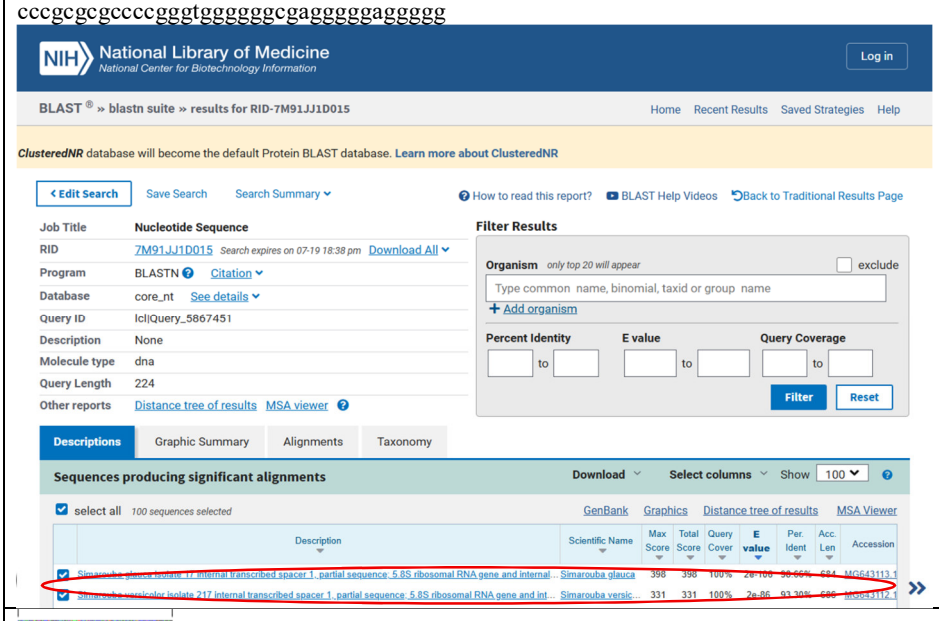


SCRIPCOGSG82

Fig 12. Chromatograms of *Simarouba glauca*

Table 11: Sequence obtained *Simarouba glauca*

Sample code	Sequence with Blast hit and Barcode
> SCRIPCOGSG82	Agcgagcgccacagagacggcgcgtaagggtcctgggagcccccgttcaggggcgggcgcacacgacgggagctcgatgaaaaagattttcgccaccgatcgtcgcggcgaccgccgccgaggactcggatttgggccaaccgcgagcagggagcgcacgggaggccagtatccgccccgcgcctcccgcgcgccccgggtgggggggcgaggggggaggggg



CONCLUSION

This study is the first to report taxometric analysis and DNA barcoding for *Simarouba glauca*, collected in the area of Nazereth, Thoothukudi, Tamil nadu. Taxometric analysis offers a valuable tool for differentiating *Simarouba glauca* from other species within the *Simarouba* genus, while DNA barcoding enables precise species-level identification in comparison to other medicinal plants, providing novel insights into its taxonomic positioning and molecular identity. Macroscopical and microscopical examinations remain essential for the

identification of *Simarouba glauca* in both whole and powdered forms. Supplementary analyses-including powder microscopy, physicochemical evaluation, fluorescence analysis, and preliminary phytochemical screening-support the authentication and characterization of the plant by revealing its diagnostic features and bioactive phytoconstituents. These integrated pharmacognostical, taxometric, and genetic approaches not only enhance the reliability of plant identification but also establish comprehensive reference standards that can support future research and the development of pharmacopoeial monographs.

REFERENCE

1. Cronquist A, the old and the new in taxonomy. *Bull Torrey Bot club.*71,1944: 226- 246.
2. ICRAF Agroforestry Tree Database, http://ecocrop.fao.org/ecocrop/srv/en/cropView?i d=9785 2007.
3. Juyal, S. P., Singh, R. V., Lal, U. M., and Singh, J. P. in *strategies* for *making India self- reliant in vegetable oils* (eds. Rangarao V and Prasad, M.V.R.). Directorate of oil seed Research, Hyderabad, 1991, PP 335-377.
4. Bhagmal (1994) in *sustainability in oil seed* (ed.Prasad M.V.R.) Indian society of oil seed Research, Hyderabad, PP 83-90
5. Rath T. *Simarouba glauca*- an exotic edible oilseed. Indian *forester,*113(1),1987:73-76.
6. Caceres, A., Cano, O., Samayou, B, Aguilar, L., J.ethnc. Pharmacol.30(1),1990:55-73.
7. Hingu B, Sake S, Gupta H, Vyas N, Thakur K S, Thakur M. Study on the phytochemical, antioxidant and *in vitro* anticancer activity on root extract of *Simarouba glauca* DC. Plant Science Today.10(3),2023: 98–105. https:// doi.org/10.14719/pst.2481.
8. Sajeeda N, Kolgi RR, Shivakumara SL, Shivaraj Y, Karigar CS. Comparative phytochemical profile and antioxidant property of bark, flowers and leaves extracts of *Simarouba glauca*. Asian J Pharm Clin Res.12(9),2019:56-63.
9. Nagaraj N, Hegde V, Gowda SK, Achur RN, Thippeswamy NB. Phytochemical analysis of *Simarouba glauca* DC and its antibacterial activity against MDR Salmonella Typhi. Journal of Pharmaceutical Sciences and Research.13(6),2021:351-6.
10. Mikawlrawng K, Kaushik S, Pushker A, Kumar S, Kameshwor M, Sharma G. Comparative in vitro antifungal activities of *Simarouba glauca* against Fusarium oxysporum and Aspergillus parasiticus. J. Med. Plant Studies.2,2014:1-7.
11. Santhosh SK, Venugopal A, Radhakrishnan MC. Study on the phytochemical, antibacterial and antioxidant activities of *Simarouba glauca*. South Indian Journal of Biological Sciences. 2(1),2016:119-24.
12. Shalal OS, Irayyif SM. Evaluation of cytotoxicity and Apoptotic effects of *Simarouba glauca* on the Prostate Cancer Cell Lines PC3. *J pak Med Assoc.*73 (9 Suppl 8), 2023: s113-s118.doi: 10.47391/JPMA.IO024.
13. Giridasappa A, Ismail SM, Rangappa D, Shanubhoganahalli Maheshwarappa G, Marilingaiah NR, Gollapalli SS, Daddakunche Shivaramu P. Antioxidant, antiproliferative and antihemolytic properties of phytofabricated silver nanoparticles using *Simarouba glauca* and Celastrus paniculatus extracts. Applied Nanoscience.11,2021:2561-76.
14. Jose SP, Sukumaran S, Mohanan R, Saji S, Asish A, George SM. Anti-inflammatory Effect of Squalene Isolated from *Simarouba glauca* in Experimental Animal Model. Pharmacognosy Research.15(4),2023: 658-666.
15. Rudraswamy S, Hombarvali JS, Kenganora M, Doggalli N, Godhi BS, Srinivas S. Antimicrobial Activity of *Simarouba glauca* Leaf Extracts Against Oral Pathogens− An In Vitro Study. Journal of Orofacial Sciences.15(1),2023:8-15.
16. Franssen FF, Smeijsters LJ, Berger I, Medinilla Aldana BE. In vivo and in vitro anti-plasmodial activities of some plants traditionally used in Guatemala against malaria. Antimicrobial agents and Chemotherapy.41(7),1997:1500-3.
17. Wright CW, O'neill MJ, Phillipson JD, Warhurst DC. Use of microdilution to assess in vitro antiamoebic activities of Brucea javanica fruits, *Simarouba glauca* stem, and a number of quassinoids. Antimicrobial Agents and Chemotherapy.32(11),1988:1725-9.
18. Ridahunlang N. Evaluation of Leaf Extracts of *Simarouba Glauca* on Experimentally Induced Inflammatory Bowel Diseases in Wistar Rats (Doctoral dissertation, KMCH College of Pharmacy, Coimbatore).
19. John PP, Jose N, Carla Sr B. Preliminary pharmacological screening of *Simarouba glauca* DC leaf extracts for hepatoprotective activity. World J Pharm Pharmaceutical Sci. 5,2016:1714-24.
20. Hussain MS, Mohit, Khan MD. Pharmacological uses of *Simarouba glauca*: a review. plant Archives.21(1),2021: 648-655.

21. Lakshmi KS, Sangeetha D, Sivamani S, Tamilarasan M, Rajesh TP, Anandraj B. In vitro antibacterial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of *Simarouba glauca* leaves extracts. International Journal of Pharmaceutical Sciences and Research (IJPSR). 5(2),2014:432-7.
22. Santhana Bharathi N, Mahendran P. Exploration of acaricidal properties of certain seed oils against the Red Spider Mite (RSM), Oligonychus coffeae Nietner (Tetranychidae: Acarina) infesting tea. J Entomol Zool Stud. 9(4),2021:297-301.
23. Osagie-Eweka SD, Orhue NE, Amaechina FC, Omogbai EK, Moke EG. Preliminary Investigative Study on the Blood Pressure- Lowering Potential of Aqueous Leaf Extract of *Simarouba glauca* (AESG) on Normotensive Adult Wistar Rats. Biology, Medicine, & Natural Product Chemistry.12(1),2023:1-4.
24. Pawar KR, Janavale GB, Wagh SG, Panche AN, Daspute AA, Pohare MB, Harke SN. Phytochemical analysis of *Simarouba glauca* Dc. and Comparison of its Bioactivity. Asian Journal of Immunology. 2019 Dec 26:1-1.
25. Antony J, Thomas A, Gnanasekaran D. Review study on pharmacological importance of *Simarouba glauca*. International Journal of New Technology and Research. 2(10),2016:263412.
26. Prajapati C, Reddy M, Bhatt M. Evaluation of anticancer activity using leaf extract of *Simarouba glauca* on leukemic cancer cell lines. Evaluation.3(2),2018:52-6.
27. Vikas B, Kunjiraman S, Rajam SS, Anil S. The Apoptotic Properties of Leaf Extracts of *Simarouba glauca* against Human Leukemic Cancer Cells. Asian Pacific Journal of Cancer Prevention: APJCP. 22(4),2021:1305.
28. Hajjar, D., Kremb, S., Sioud, S., Emwas, A., Voolstra, C., & Ravasi, T. Anti-cancer agents in Saudi Arabian herbals revealed by automated high-content imaging. PLoS One, 12(6), 2017. e0177316. https://doi.org/10.1371/journal.pone.0177316.
29. Wallis TE. Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs. Third Edition. Boston: Little, Brown and Company; 1965.
30. Fahn A. Plant anatomy. Third Edition Pergamon Press, Oxford; 1980.
31. Kannan N, Gopi DK, Shanmugam SK, Narayana SK, Shamsudin AK. Comparative macro-microscopic studies of three wild Solanum species (*S. melongena* L., *S. incanum* L. and *S. virginianum* L.). J Res Siddha Med. 6,2023:1-10.
32. Kokoshi CJ and Kokoshi RJ, Sharma PJ 1958 Fluoresence of powder vegetables drugs under UV radiation. J Am. Pharm.Assoc. 47 (10), 1958:715-717. Doi: 10.1002/jps.3030471010.
33. Anonymous authors, The Ayurvedic pharmacopoeia of India. Edn 1 part 1, Vol.2, Government of india, New Delhi, 1986, pp:189-196.
34. Bentley and driver's Text book of phrmacutical chemistry. Edn 8, Revised by L.M.Atherden, 1981, pp:174-176.
35. J. B. Harbone, Phytochemical methods A guide to modern techniques of plant analysis. edn 3, 1998.
36. Seo, Ji-Hye, Jung-Eun Kim, Jung-Hyun Shim, Goo Yoon, Mi-Ae Bang, Chun-Sik Bae, Kyung-Jin Lee, Dae-Hun Park and Seung-Sik Cho, HPLC analysis, optimization of Extraction conditions and biological evaluation of Corylopsis coreana Uyeki Flos. Molecules.2016; 21(1): 94.
37. [(accessed on 21 December 2020)]; Available online: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf.
38. Singleton VL, Orthofer R, Lamuela-Raventos R.M. Analysis of Total Phenols and other Oxidation Substrates and Anti-oxidants by means of Folin- Ciocalteu reagent Methods: Enzymol: 1999, 152-178.
39. Boham AB, Kocipai AC. Flavonoid and condensed tannins from Leaves of Hawaian Vaccininum vaticulum and Viacalycinium: Pacific Science: 48, 1974; 458-463.
40. Indumathi C, Durgadevi G, Nithyavani S, Gayathri PK. Estimation of terpenoid content and its antimicrobial property in Enicostemma littorale. Int J Chem Tech Res.6(9),2014:42647.
41. Ajanal M, Gundkalle MB, Nayak SU. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Sci Life.31(4),2012:198–201. doi:10.4103/0257-7941.107361.
42. Sneath peter, Sokal RR. Numerical taxonomy: the principles and practice of numerical classification. San Francisco: W.H. Freeman; 1973. p.41-66.
43. de vere N, Rich TC, Trinder SA, Long C, DNA barcoding for plants. Plant Genotyping: Methods and protocols. 2015: 101-108.
44. https://blast.ncbi.nlm.nih.gov/blast.cgi.