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Pharmacognostical evaluation of the leaves of *Canavalia gladiata* (JACQ.) DC

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Abstract

Canavalia gladiata (Jacq.*)* DC belongs to the family Papilionaceae. The leaves of *Canavalia gladiata* were pharmacognostical investigated in terms of macroscopic, microscopic and physico-chemical parameters including extract values and ash values determination. The phytochemical screening of different leaves extract disclose the presence of various phytoconstituents including alkaloids, glycosides, flavonoids, carbohydrate, proteins, amino acid, phenols and tannins will aid for upcoming investigation in their pharmacological analyses.

Keywords: *Canavalia gladiata* leaves, pharmacognostical, phytochemical, physico-chemical

Introduction

Canavalia gladiata is commonly called as Sword bean, Jack bean or Beach bean which belongs to the Papilionaceae family and is widely distributed in China, Japan, Eastern and Western Ghats of South India [1]. *Canavalia gladiata* is an annual perennial climbing plant grown vigorous, deep-root, slightly woody and branches. The stems are glabrous which can grow upto10 meters long and twining into other plants for support. Grows well in lowland tropical areas, succeeding at the altitude up to 1,500 meters. *Canavalia gladiata* prefers temperatures are within the range of 15-30 °c and the annual rainfall in the range of 800-1,800mm, it can also tolerate range from 12-36°c and 600-2,600mm. The flowers are white or ilac, in lax few flowered curved axillary racemes. The plant *Canavalia gladiata* prefers moist soil and can fix Nitrogen itself. The presence of numerous bioactive plant metabolites like alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponins, flavonoids, steroids, and phenolic compounds have wide range of significance and used in variety of applications. In japans traditional herbal medicine *Canavalia gladiata* is used in the treatment of vomiting, belly whopper, kidney disorders, asthma, dysentery, neurodegenerative disorders, schizophrenia, inflammatory diseases and cancers [2-3].

Fig 1: *Canavalia gladiata* leaves

Materials and Methods

Collection, identification and authentication of the plant

The fresh leaves were collected in the Kottipallathu Oodi, Salem (DT), and TamilNadu in the month of December 2020. Finally identified and validated by Dr. R. Jayaraman Ph.D., as *Canavalia gladiata* in the Institute of Herbal Botany Plant Anatomy Research Centre and Chennai.

Macroscopical studies

The fresh leaves of *Canavalia gladiata* were observed the different organoleptic property like shape, size, color, odor and taste which is useful in the qualitative control of the crude drug [1, 4].

Microscopical studies

Collection of specimen

The leaves are collected from plant and cut into the pieces. These specimens are fixed for 24 hours in FAA (Formalin-5ml+Acetic acid-5ml+70% Ethyl alcohol-90ml) and dehydrated with tertiary Butyl alcohol **[5]**. To attained super saturation of thiobarbituric acid solution gradual addition of paraffin wax were carried out. The specimens were cast into paraffin blocks.

Sectioning

The paraffin plant specimens were sectioned with the help of Rotary Microtome and thickness of the sections was 10-12 µm. Toluidine blue were used to stain the section.

The section were stained with safranin, iodine for starch, dye rendered pink color to the cellulose walls, blue to the lignified cells, violet to the mucilage, blue to the protein bodies etc. [6, 7].

For studying the morphological, patterning and trichomes distribution, paradermal sections as well as clearing of leaf with 5% sodium hydroxide (NAOH) **[6]**. Powered materials of different cell component were cleared with sodium hydroxide and mounted in glycerin medium after staining.

Photomicrographs

Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit and figures are specified by scale bars. For the study of starch grains, crystals and lignified cells polarized light were employed. Bright field was used for the normal observation. Structures have bipolar property; they appear bright against dark background under polarized light [8-9].

Powder microscopy

The leaves of *Canavalia gladiata* were shade dried and pulverized, then the powder was passed through the sieve no.60 which is used for powder analysis and organoleptic nature such as color, odor and taste were examined with the use of different staining reagents that is 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid (HCL) and N/50 iodine. Slides were observed under the microscope [11].

Physico-chemical studies

The different physicochemical parameters were determined which involves Alcohol and water soluble extractive values, total ash value, loss on drying, water soluble ash, acid insoluble ash etc. [11].

Preparation of extract

The *Canavalia gladiata* leaves were collected and shade dried. Then pulverized with a mechanical grinder followed by passing the powder through different sized sieve and stored in an airtight container for future use.

Soxhlet extraction is the process of continuous extraction method the coarse powder material (100 g) was extracted using suitable solvent [12]. The process involves for extraction followed by evaporation of solvent in the increasing polarity. The solvent was removed by distillation method under reduced pressure. The greenish sticky residue is obtained (yield 10%w/w with respect to the plant material). The concentrated crude extract was stored and used for the upcoming studies.

Preliminary phytochemical screening

The different solvent extracts obtained from various successive solvent extractions were then subjected to qualitative chemical analysis to find out the presence of plant constituents like saponin, proteins, alkaloids, phenolics, glycosides, carbohydrates and tannins, amino acids, phytosterols [11].

Result and Discussion

Macroscopic evaluation

Organoleptic characters on the leaves of *Canavalia gladiata were* carried out

Color: Green

Odor: Slightly bitter

Taste: Characteristic

Shape: Alternate, large, trifoliate

Type: Oval

Length: 7.5-20 cm long x 5-14 cm broad

Fig 2: Different sized *Canavalia gladiata* leaves

Microscopic evaluation

Anatomy of the leaf

In the transverse section (TS) of *Canavalia gladiata* leaf shows thick and prominent midrib (Fig 3). Midrib consists of Adaxial wide raised hump is 50mm wide and 30µm in height and abaxial part has wide and thick midrib is 16.5 mm perpendicular plane. The abaxial part is 15 µm in horizontal plane and 10 mm in perpendicular plane. The midrib consists of uniformly thin rectangular small thick walled epidermal cells which are followed inside by thin layer of rectangular hypodermal cells. The Adaxial hump occurs as a thick vertical segment of fiber (fig: 4).

The Adaxial vascular bundle consists of cluster of wide circular xylem elements with small group of phloem elements on the upper and lower side. The major vascular segment in the midrib is wide deeply curved with several radial lines of circular thick walled compact xylem elements. Phloem elements occur in discrete circular units both on the abaxial part and adaxial part. A thick arc of sclerenchyma cells occur on the lower part of the vascular arc (fig: 4). the ground tissue is wide and well preserved on the lower part of the midrib. The Calcium oxalate crystals occur in single and occupying entire cell lumen with prismatic type occurs in the cells bordering the vascular segment (fig: 5).

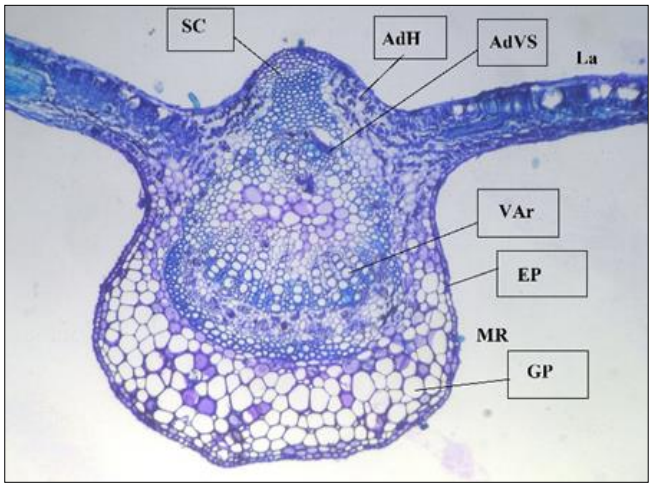

Fig 3: TS of leaf through midrib

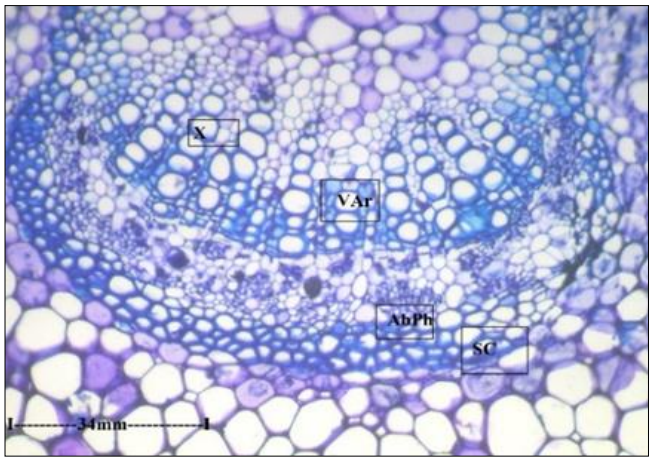

Fig 4: TS of midrib enlarged AdH -Adaxial Hump, Advs.-Adaxial vascular strand, Ep-Epidermis, Gp-Ground parenchyma La-Lamina, MR-Mid Rib, SC-Sclerenchyma, VAr-Vascular Arc, X-xylem, Abph-Abaxial phloem.

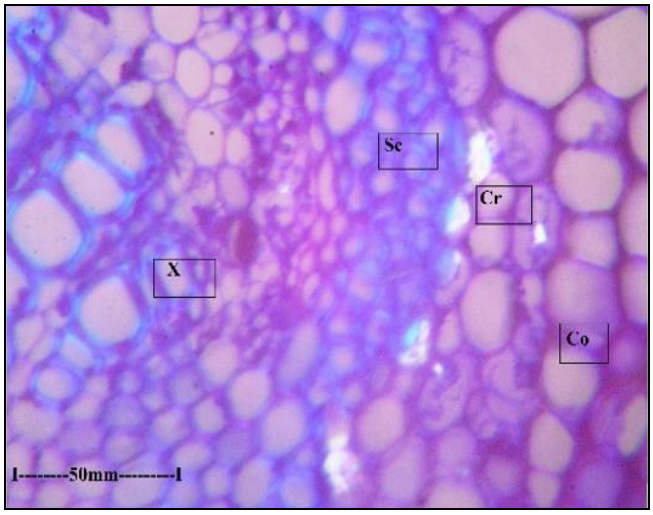

Fig 5: Calcium oxalate crystals in the cells outer to the vascular bundles X-Xylem, SC-Sclerenchyma, Cr-Crystal, Co-cortex

Lateral vein

The lateral vein is 550µm vertical plane which is Plano convex with slightly raised adaxial side and deeply convex abaxial part (fig: 6).The lateral vein consists of a thick circular collateral vascular strand. The lateral vein consists of vertical rows of xylem elements with Adaxial and abaxial layers of phloem. Sclerenchyma elements occur in thick horizontal blocks, both on the upper and lower sides of the vascular bundle. The epidermal cells are thin thick walled cell. There is a sub epidermal layer inner to the epidermal cell. The ground parenchyma consists of wide angular compact parenchyma cells.

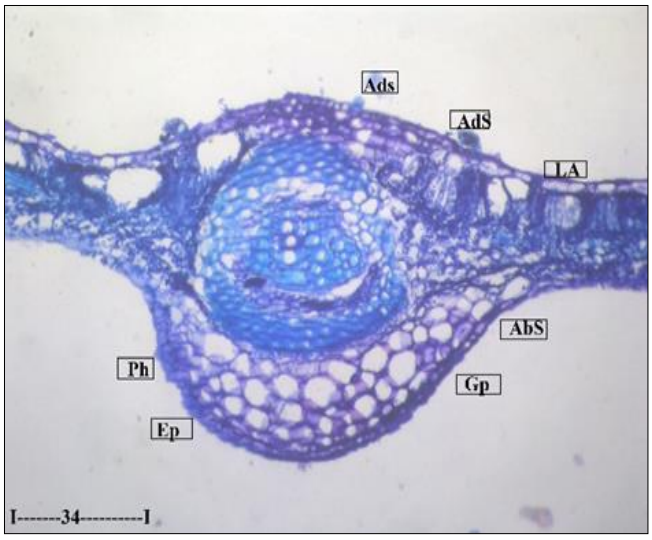

Fig 6: TS of lateral vein Abs-Abaxial side, Ads-Adaxial side, GP-Ground Parenchyma, La-Lamina, Ph.-Phloem, Ep-Epidermis.

Lamina

The lamina is 300µm thick, dorsiventral with adaxial palisade and abaxial spongy mesophyll tissue. The palisade mesophyll tissue are vertically elongated long and thin The adaxial epidermal layer is thin walled and abaxial epidermal cells are small with four angular.

Spongy mesophyll tissue includes small lobed loosely arranged parenchyma cells are seen in vascular strands

Fig 7

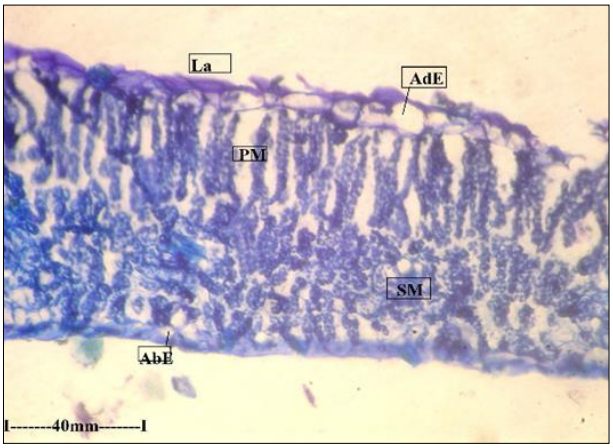

Fig 7: TS of lamina AbE-Abaxial Epidermis, AdE-Adaxial Epidermis, PM-Palisade Mesophyll tissue, SM-Spongy Mesophyll tissue, La-Lamina.

Leaf margin

Leaf margin is 200µm thick. The Adaxial epidermis consists slightly narrow marginal part and wide vertically oblong thin walled parenchyma cells. The mesophyll tissues are not well differentiated in the leaf margin. Vascular strand is seen in the sub marginal region (fig: 8)

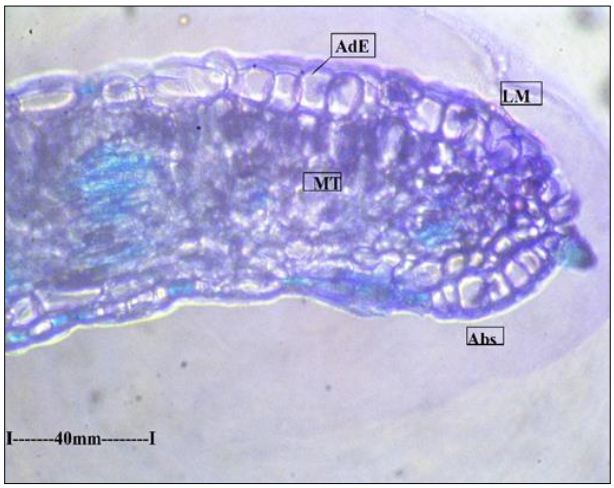

Fig 8: TS of lateral vein MT-Mesophyll tissue, AbE-Abaxial Epidermis, AdE-Adaxial Epidermis, LM-Leaf margin

TS of petiole

The transverse section of petiole is circular in cross sectional view and 1.4 mm in diameter. The Adaxial side is slightly concave the petiole consists of epidermis (small rectangular and thin walled), ground parenchyma (homogenous with compact angular parenchyma cell), vascular strands (Fig 9) the vascular strands are located in the center. The strand is deeply grooved along the adaxial side. So that vascular bundles appear bilobed. Xylem tissue consists of scattered numerous xylem elements and xylem fibers. The phloem occurs in thick layers all around xylem strands. The xylem strands of petiole includes numerous, circular vessel elements with circular thin walled cells. The ground tissue of the xylem strand consists of xylem fibers. Phloem occurs all around the lobed xylem segments. (Fig: 10). The Calcium oxalate crystals are outer to the phloem which appear to the prismatic type and they are elongated bodies mostly closely aggregate together. (Fig: 11).

Fig 9: TS of petiole entire view

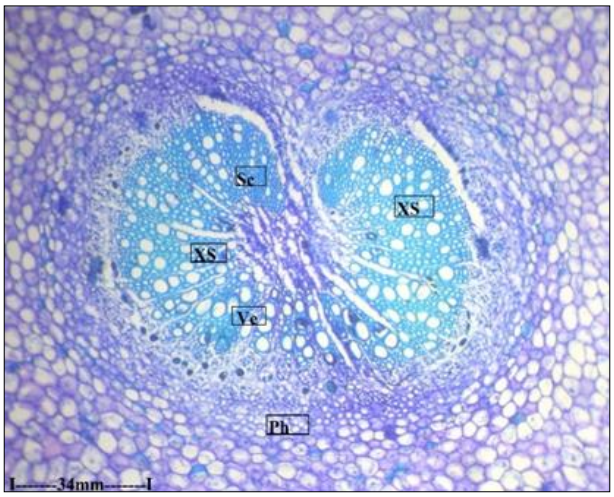

Fig 10: The vascular segment enlarged Ads-Adaxial side, Ep-Epidermis, GP-Ground Parenchyma, Ph-Phloem, VS-Vascular Strand, XS-Xylem segment, Cr-Crystals, SC-Sclerenchyma, Ve-Vessel, X-Xylem.

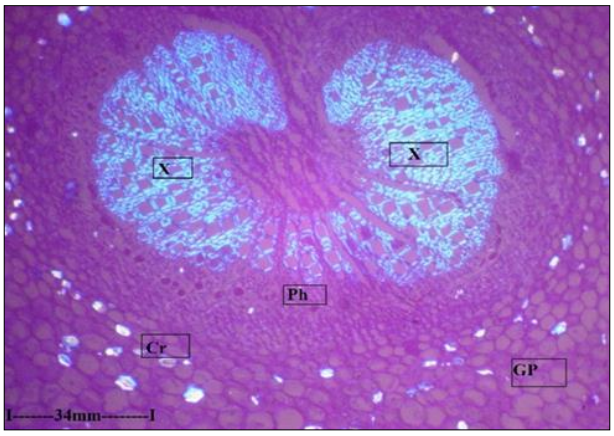

Fig 11: Crystals distribution in the ground parenchyma outside phloem zone GP-Ground Parenchyma, Ph-Phloem, XS-Xylem segment, Cr-Crystals, X-Xylem.

Powder microscopy

The investigation of dried powder of *Canavalia gladiata* leaf showed elongated and non-lignified fiber (Fig: 12), xylem vessels (Fig: 13), uniseriate multicellular trichomes (Fig: 14).

Fig 12: Elongated and non-lignified fiber

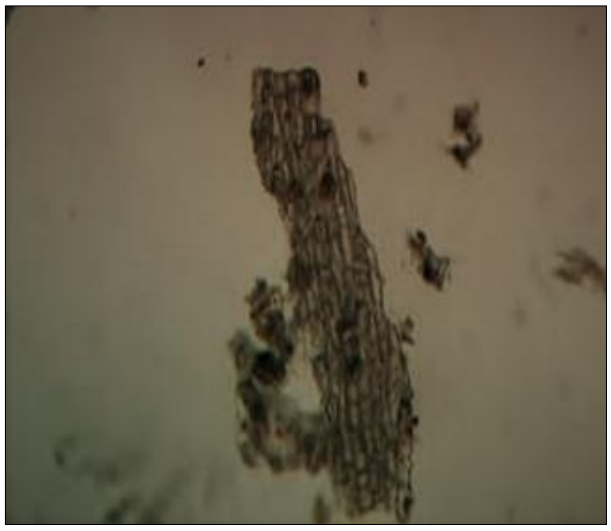

Fig 13: xylem vessels

Fig 14: Uniseriate multicellular trichomes

Physicochemical investigations

The physicochemical investigation of crude powder of *Canavalia gladiata leaves* is shows the average values are expressed as percentage of shade-dried material. It involves the different parameters like loss on drying, total ash values, acid insoluble ash, alcohol and water soluble extractive values.

Table 1: Physicochemical parameters of powder of *Canavalia gladiata* leaves

S.No.	Physicochemical Parameters	Average value %w/w
1.	Loss on drying	1.31
2.	Total ash value	22.35
3.	Acid insoluble ash	8.37
4.	Water soluble ash	5.1
5.	Alcohol soluble extractive value	2.52
6.	Water soluble extractive value	8.68

Phytochemical investigation

Extraction of plant material

The yield of various successive extracts of powdered leaves are given in table 2

Table 2: Different extraction of plant material

S. No.	Extract	Yield (%w/w)
1.	Petroleum ether	2.35
2.	Chloroform	2.76
3.	Ethyl acetate	3.04
4.	Alcohol	16.45
5.	Water	18.01

Preliminary phytochemical screening

The phytochemical screening revealed the presence of secondary plant metabolites which includes Alkaloids, carbohydrates, phenolics, tannins, proteins, amino acids, saponin and phytosterols are shown in table 3.

Table 3: Phytochemical screening on different extracts of *Canavalia gladiata*

S. no.	Chemical constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids	-	+	+	+	-
2.	Glycosides	+	+	+	+	-
3.	Steroids	+	+	+	+	-
4.	Flavonoids	-	-	-	+	-
5.	Carbohydrates	-	+	-	+	+
6.	Tannin and phenolic compound	-	-	-	-	-
7.	Proteins	-	-	+	+	-
8.	Amino acid	-	-	+	+	-
9.	Gum and mucilage	-	-	-	-	+

(+) presence (-) Absence

Results and Discussion

The suitability of a crude drug is determined by the Sensory evaluation and qualitative evaluation based on the observation of morphological and sensory profile. In this report, the different morphological, microscopically, physicochemical standards have been developed. This study plays an important role in the developing standards for identification, quality and purity of *Canavalia gladiata* leaves. The validation of crude drug is done by observation of cellular level morphology. Microscopic evaluation is one of the simply and important methods for the identification of the correct source of the plant materials like compact xylem elements, Phloem elements occur in circular units, Sclerenchyma cells occur on the vascular arc, Calcium oxalate crystal of prismatic type occur in the vascular segment.. Epidermal cells are rectangular and thin walled.

The ground parenchyma is compact with angular parenchyma cells. The macroscopic characters of the leaf can serve as diagnostic parameters. The ashes values are used to determine the presence of foreign inorganic matters such as metallic salts and or silica. Acid insoluble ash identifies the adulteration due do dirt, sand (or) soil of crude drug. The exhausted or adulterated drugs are identified by using extractive values. Phytochemical evaluation plays an important role in the discovery of drug. The phytochemical analysis showed the presence of various plant constituents. The dried powder of the *Canavalia gladiata* leaf revealed the presences of elongated and non-lignified fiber, xylem vessels and uniseriate multicellular trichomes.

Conclusion

In that investigation, the detailed pharmacognostical description is given by which encompass macroscopic and microscopic characters which helpful for the botanical identification of the leaves of *Canavalia gladiata* (Jacq.) DC. The macroscopic examination of the leaf exhibited the color, shape, size etc. In microscopic perusal of the leaf revealed epidermis, lamina, midrib, mesophyll, stomata, calcium oxalate crystal. The quantitative analysis of different phytoconstituents shows detailed information which is helpful for the future invention in isolation of new active compounds and valuable for the various pharmacological works. From the present study helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

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