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Standardization and phytochemical evaluation of *Cassia siamea* leaves

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Abstract
Background: Cassia siamea belonging to the family Caesalpiniaceae have been used widely in ethnomedicinal practices for its medicinal value.
Objective: The objective of the study was to evaluate Standardisation parameters for Leaves of C. siamea Plant and Phytochemical evaluation of Petroleum Ether Extract, Chloroform extract and Methanol Extract of Leaves of same plant.
Materials and Methods: Evaluation of leaf samples was performed by determining morphological, microscopical characters, physicochemical, and phytochemical analysis as per the World Health Organization Guidelines.
Results: Morphologically, Leaves were alternate, pinnately compound, with 6 to 12 pairs of leaflets. Microscopically, Anomocytic and Paracytic stomatas were found only in the abaxial surface C. Siamea. Phloem was encircled by xylem. Lower epidermis shows presence of unicellular covering trichomes. Physicochemical analysis and phytochemical analysis resulted in a valuable data to establish standards for the plant.
Conclusion: The establishment of pharmacognostical, Physicochemical and Phytochemical parameters of leaf material will be useful in identification and standardization of C. siamea in obtaining quality formulations.

Keywords: *Cassia Siamea*, standardization, phytochemical analysis

Introduction
Cassia siamea is a tropical plant of family Caesalpiniaceae and is native of South-east Asia. It is well-known for its nutritional, medicinal and economic importance and has been traditionally used for the treatment of ailments such as fever, malaria, diabetes, hypertension, asthma, constipation, diuresis, CNS disorders, jaundice, abdominal pain and menstrual pain either in crude form or as decoction of leaves and flowers. In some part of Asia, leaves and flowers of C. siamea are used as vegetables. C. siamea plant is found to be medicinally important for the treatment of several pathological complications. At the same time, it has also nutritional and economic importance. The major phytochemical constituents present include anthraquinones along with coumarins, alkaloids, flavonoids, glycosides, tri-terpenoids, sterols and other polyphenols. Further research on this plant has explored its specific pharmacological properties such as analgesic, antioxidant, anti-inflammatory, anti-malarial, vasorelaxant, anti-tumor, anti-arrhythmic, anti-androgen, anti-HIV and anxiolytic activity in various in- vitro and studies. There is couple of reports that show toxicity concerns of this plant [1, 2, 3, 4].

Materials and Methods
Plant collection and identification
The whole plant of *Cassia siamea* was collected from Nashik district (Maharashtra) during flowering season. Herbarium specimen of the plant was deposited in Botanical Survey of India, Pune and it was identified by the botanist P. A. Ingle (Scientist B). (Authentication No. BSI/WRC/IDEN.CER./2018/H2/89).

Preparation of powder
The leaves were washed thoroughly for about 2-3 times in running tap water to remove the impurities like soil particles and adhered debris and finally with distilled water. The leaves were shade dried, subjected to pulverization and powder samples were passed through sieve # 60. Standardization of this powdered sample was carried out.

Preparation of plant extract
The dried powdered leaves were extracted successively with the increasing order of polarity by using Soxhlet extraction process and filtered. The filtrate was evaporated near to dryness under reduced pressure to yield dry extract and utilized for further assessments.

Pharmacognostic Evaluation
Macroscopic evaluation
Macroscopic characters like size, shape, apex, margin venation, base, petiole, surface, and color of leaves of C. Siamea were studied per visual observation, following the standard procedure of taxonomy and verified.

Microscopical evaluation
For microscopical studies, the fresh leaves of C. Siamea were removed from the plant and transverse sections were obtained. The sections were cleared with chloral hydrate solution, washed in distilled water, and then stained with Phloroglucinol-hydrochloric acid (1:1). Section was mounted on slide with the help of glycerin and analyzed by light microscope. The photographs of the section were taken using the digital camera [8, 10].

Quantitative microscopical evaluation
The leaf microscopic characters like Palisade ratio, stomatal number, stomatal index, vein islet number and vein termination number were determined.

Physicochemical evaluation
According to the WHO guidelines (2002), physicochemical parameters were analyzed for the powdered dried leaves of

C. siamea. Loss on drying, ash values and extractive parameters such as alcohol-soluble extractive and water-soluble extractive values were [10] determined. All the samples were analyzed in triplicate.

Preliminary phytochemical evaluation

The air-dried leaves of C. Siamea were made into coarse powder. The powdered material was extracted successively with the increasing order of polarity by using Soxhlet extraction process and filtered. The filtrate was evaporated near to dryness under reduced pressure to yield dry extract and it was subjected to phytochemical analysis to detect the secondary [11] metabolites according to standard procedures.

Results

Pharmacognostic evaluation

Macroscopic evaluation

The leaves were almost 22-30 cm long with greenish-yellow Color and were alternate and pinnately compound. 6-12 pairs of leaflets were present on short stalk (3-6 cm long, 11-20 mm wide) that is rounded at both the end.(Fig. 1 and Fig. 2) The leaves were having characteristic odor and slightly bitter in taste.

Fig 1: C. Siamea Whole Plant

Fig 2: Leaves of C. Siamea

Microscopical evaluation

Microscopic examination of the leaves (Fig.3) showed cuticle, epidermis, palisade cells, collenchymatous and parenchymatous cells in single or in groups. Transverse section of leave was circular in outline. Epidermal cells were barrel shaped having cuticle in outline. Externally cutical layer is present, made up of thick layer of cells followed by epidermal layer, shows presence Anomocytic stomata. Parenchymatous cells towards the epidermis were smaller in size while those towards the center were bigger. Parenchymatous cells had sufficient inter-cellular spaces. In transverse section, the epidermal cells are tangentially elongated. The cells of the lower epidermis are somewhat rectangular in shape and arched outside and are smaller than those of the upper epidermis. The epidermal cells of the margin region show uniformity in shape and are smaller in dimension.

Cells below the epidermis in the margin region are collenchymatous. The palisade tissue consists of one and, at places, two layers of closely packed cells. The spongy mesophyll present beneath the palisade layer consists of 7 to 10 layers of loosely arranged cells. Chloroplasts are present in abundance in the mesophyll cells. The bigger veins have a structure resembling that of the midrib and the phloem surrounds the xylem dorsally. A transverse section through midrib region shows a prominent projection on the lower surface. Above the lower epidermis there are one to two layers of collenchymatous cells followed by 8 to 12 layers of parenchymatous cells with intercellular spaces. The parenchyma cells become gradually smaller in the inner region adjacent to vascular tissue.

At middle side 6-8 layers of spongy parenchyma, vascular bundle, Fibers, vessels and trachoid's were observed. The xylem is divided into separate bundles, but the phloem and the pericyclic fibers are in a continuous ring. A pair of secondary vascular bundles lies laterally to the adaxial bundle. Vascular elements showed scalariform and spiral thickening. Upper and Lower epidermis shows presence of unicellular covering trichomes. The cortical sclerenchyma and trichomes are absent. Cluster crystals are confined to the pith cells.

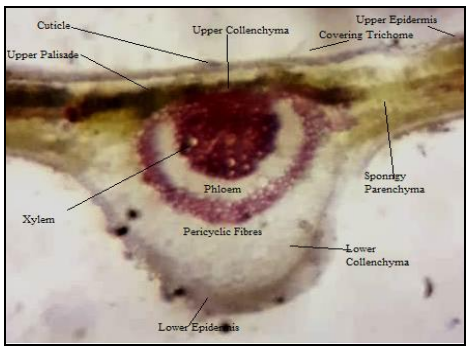

Fig 3: Transverse section of C. Siamea Leaves

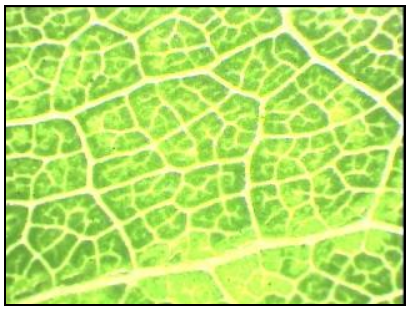

Fig 4: Vein islet number and vein termination number

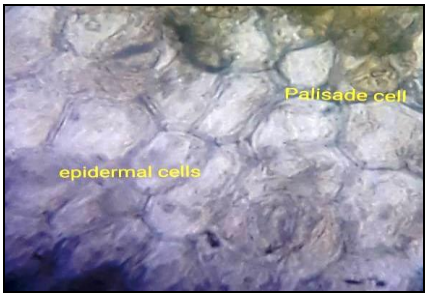

Fig 5: Paracytic Stomata

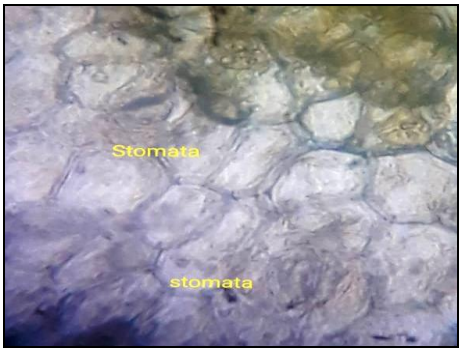

Fig 6: Anomocytic stomata

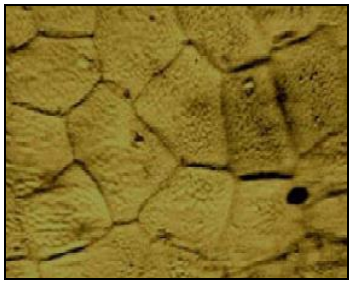

Fig 7: Adaxial Surface

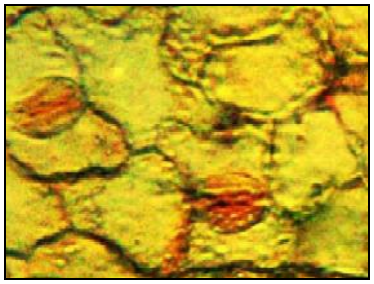

Fig 8: Abaxial Surface

C. siamea bears hypostomatic leaves i.e. stomata present only at the abaxial surface (Fig. 8) of leaves. Here it was found that the shape of epidermal cells varies in their surfaces. The adaxial surface (Fig. 7) of the leaf shows tetragonal to polygonal shaped epidermal cells but the abaxial surface i.e. stomata bearing surface shows undulating to irregular shaped epidermal cells. Anomocytic stomata and Paracytic stomata (Fig. 5 and 6) are found only in the abaxial surface of C. siamea.

Quantitative microscopical evaluation

The leaf microscopic characters like Palisade ratio, stomatal frequency, stomatal index, vein islet number and vein termination number were observed as per Table. 1.

Table 1: Quantitative microscopy of the leaves of C. Siamea

Determinations	Range
Palisade Ratio	4-5
Stomatal frequency	440-490
Stomatal index	35-40
Vein islet number	14-19
Vein termination	15-25

Physicochemical evaluation

Physicochemical analysis of powdered C siamea leaf, namely, Extractive value, Moisture content and Ash value was observed as per Table. 2.

Table 2: Physicochemical Evaluation

Sr. no.	Parameter	Observed value
1	H_2O soluble extractive VALUE	0.64%
2	Alcohol soluble extractive value	0.28%
3	Moisture content	18.48%
4	Total ash value	0.21
5	Acid insoluble ash	0.17
6	Water soluble ash	0.20

Preliminary phytochemical evaluation

Leaf extracts obtained by successive extraction process was subjected to phytochemical analysis to detect presence of the secondary metabolites according to standard procedures. The concentration range for secondary metabolites is observed as per Table.3.

Table 3: Preliminary Phytochemical Evaluation

Name of test	Pet ether extract	Chloroform extract	Methanol
1. Tests For Carbohydrate			
Molish Test:	+	+	+
Fehling's Test	-	+	++
2. Tests For Proteins			
Biuret test:	-	+	-
Millons test		+	++
3. Test For Amino Acids			
Ninhydrin test:	-	-	-
4. Test For Steroids			
Salkowski Test	+	-	+
5. Glycosides			
Test for Saponins:	-	-	+
Legal test	-	-	+
6. Test For Alkaloids			
Wagner reagent Test	-	+	++
Mayer's Test	-	+	++
7. Test For Tannin And Phenolic Compounds			
Ferric Chloride Test	-	+	++
Dilute Nitric Acid Test	-	+	++
8. Test For Flavonoids			
Shinoda Test	-	-	+
Lead acetate test	-	-	+

Note:"+" means present in trace, "++" means present in moderate, "+++" means present in high concentration. "-" means absent

Discussions

The standardization of Herbal Medicine is important in order to assure the safety and Therapeutic efficacy of same. C,siamea leaves are having various medicinal uses. In order to achieve standardization of it, Pharmacognostic, Physicochemical and Phytochemical evaluation of C. siamea was done. In macroscopic study it was observed that Leaves were green in colour, with dimension 5.5-6 cm X 3-3.5 cm, lanceolate in shape, glabrous surface, venation pattern was pinnate.

Microscopical study showed the presence of cuticle, epidermal cell, palisade cells, xylem, Phloem, pericyclic fibers, Anomocytic and Paracytic stomata with covering trichomes. Quantitative microscopy was also performed since it is identified as characteristic and constant features of Particular species and it can be used as standardisation parameter for identification of C.siamea. Physicochemical Values will be helpful for Identification of same plant. Phytochemical evaluation has shown presence of Alkaloids

and Tannins in Moderate amount and flavonoids in Trace amount in Methanolic Extract of C,siamea Leave which indicates its probable use in various Therapeutic Conditions.

Conclusion

The study reported complete pharmacognostical parameters of leaves of C. siamea. These specifications could be used to authenticate and evaluate the quality of C. siamea plant and hence differentiating it from closely related species. This work also could be useful in compilation of a suitable monograph of C. Siamea and also contributes toward establishing the Pharmacopeial standards.

Acknowledgments

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Conflict of Interest Statement

We declare that we have no conflict of interest

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