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(RESEARCH ARTICLE)

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Micro-qualification by estimation of leaf constants of the ethnobotanical plant *Costus igneus* N.E. Br by optical microscopical analysis

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Abstract

Costus igneus N.E.Br, traditionally known as Insulin Plant, is an ethnobotanical perennial plant used by aboriginal people for treatment for Hyperglycaemia, or abundancy of sugar in body. In modern studies, researchers have found that the leaves of *Costus igneus* N.E. Br to be effective especially for Type-I Diabetes. In addition to its's Anti-Diabetic property it has various other properties like Anti-Oxidant, Anti-Urolithic, Anti-Proliferative, Anti-Inflammatory and Anti-Lipidemic property. The Isolated Pharmacologically active moiety is a Flavonoid called Quercetin. A traditional method of identification of a plant species is by estimation of its Leaf Constants. Just like fingerprints, each plant has its own unique identification in terms of leaf constant. The fresh leaves of the plant were collected from Insulin plant of Bengal School of Technology, Hooghly, Herbal Garden and a Herbarium was prepared. The Herbarium was then authenticated and identified from Botanical Survey of India, Shibpur, and West Bengal. The authenticated plant was collected for fresh leaves and the Leaf Constants that were calculated were Stomatal Number, Stomatal-Index, Palisade Ratio, Vein-Islet Number and Vein-Termination Number. The leaf constants were observed and shown in table.

Keywords: *Costus igneus* N.E.Br; Leaf Constant; Ethnobotanical; Stomata; Vein-Islet

1. Introduction

Leaf Constants by the means of Stomatal-Index, Stomatal Number, Palisade Ratio, Vein- Islet and Vein-Termination Number serves as a unique identification for each plant species. *Costus igneus* N.E. Br is a species of herbal perennial plant that generally grows in the tropical regions. Matured leaves has parallel venation and is generally 15-18 cm long. The leaves are mechanically very strong and it contains various components in form of organized drug. During microscopic studies, it has been found to contain Anomocytic Stomata. All the leaf Constants have to be estimated by making a Cross-section and Transverse-section cut. The leaves need to be soaked in water so that the plant sections can be easily obtained. Chloral Hydrate is used to eliminate the green chlorophyll. Then the sections are loaded onto a glass slide and a cover slip needs to be applied before setting up the slide on an optical microscope. Camera Lucida is used to get a one cm square of the plant part on a paper. The plant anatomy is plotted and the leaf Constants are estimated from the drawn diagram on paper. Some Leaf Constants are estimated from Transverse section while some are estimated from Cross-section. There are an estimated three lakh twenty thousand plant species found in whole world and this experimentation needs to be performed on the whole Plant Kingdom for proper identification of each plant type.

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2. Material and methods

2.1. Chemicals

- Choral Hydrate
- Demineralised Water
- Cedarwood Oil

2.2. Instruments

- Optical Binocular Microscope
- Projection Microscope
- Camera Lucida
- Micrometer (Eyepiece and Stage)

2.3. Glasswares, etc

- Beaker
- Petri Dish
- Slides
- Cover Slip
- Blades
- Peeled Potato

2.4. Stomatal Number

The average number of stomata per square millimeter of epidermis is called Stomatal Number. The Leaf was dipped in chloral hydrate solution for several hours until it lost its colour and pigments. The cube of pith was selected and vertically cut, leaf was inserted and fine sections were obtained by Transverse-Section. Fine sections mounted on glass slide with help of glycerine without any staining reagent used were placed under microscope. Under 40 times magnification the slides were observed. Using a Stage micrometer, the Eyepiece Micrometer was calibrated and a square was drawn each side 1 mm. Using computer software stomata were highlighted. Then the number of stomata was manually counted.

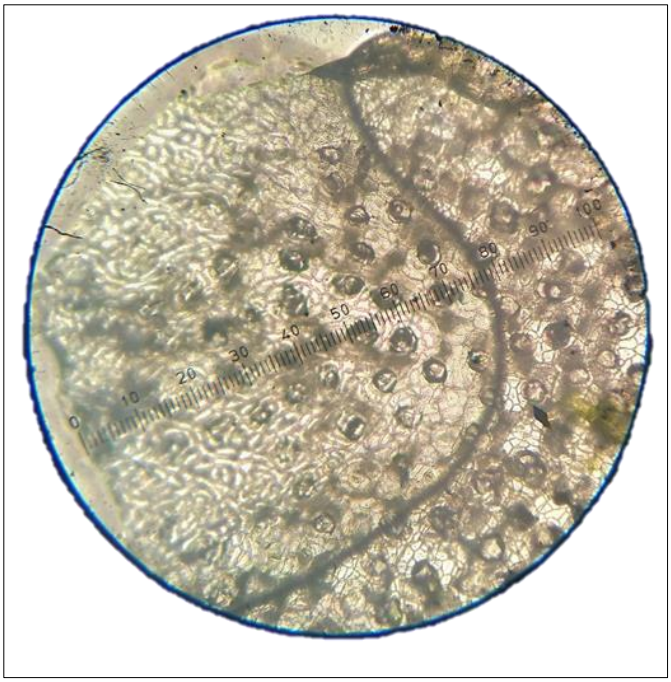

Figure 1 Microscopic observation of Transverse Section of Leaf under 40x magnification

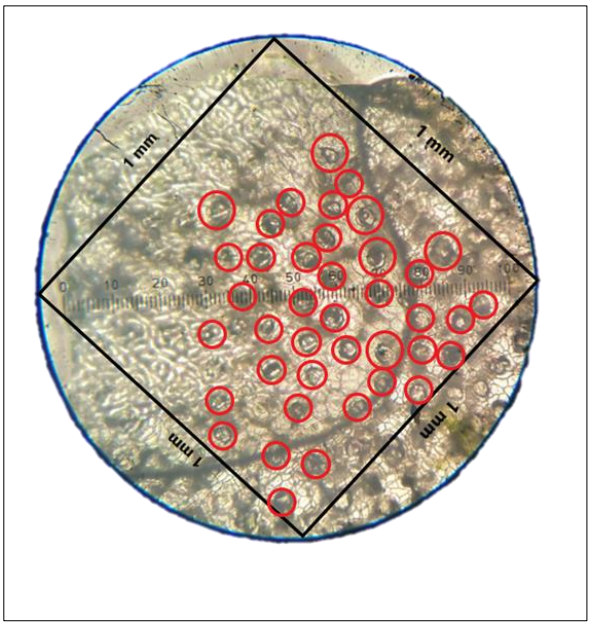

Figure 2 Extrapolated Observation for determination of Stomatal Number

The observed Stomatal Number was found to be 40. Thus, there are 40 stomata in the surface of plant leaf of an area of one square millimeter.

2.5. Stomatal Index

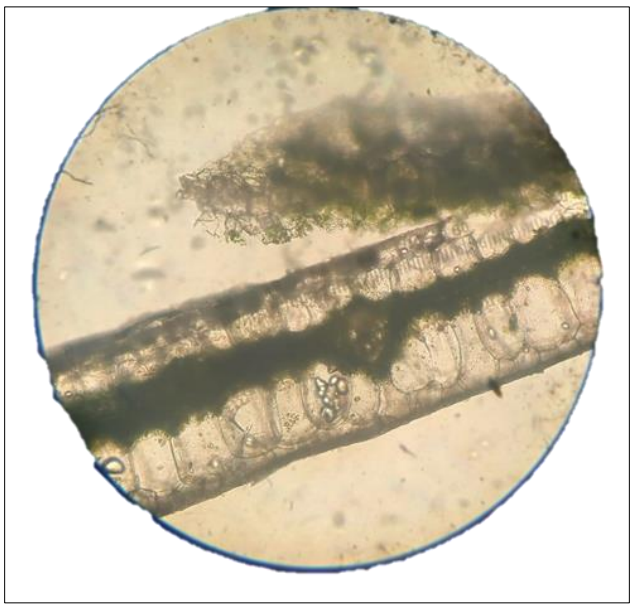

Figure 3 Microscopic observation of Cross Section of Leaf under 40x magnification

Cross section of the leaves was cut and then kept in distilled water and simultaneously in chloral hydrate. Then the pieces was mounted in a glass slide and covered with cover slip. The Slide was then observed under microscope at 40x magnification. The Stomatal Index can be calculated by the Formula Stomatal Index (%) = (S/S+E) x 100 where, S and E are the number of stomata and epidermal cells respectively in microscopic view field.

The Stomata is the bold green cell and the epidermal cells are the cells below the stomata. As in this slide there are five epidermal cells that are present adjacent to a single stoma. Thus, the observed Stomatal Index = 16.66

Figure 4 Extrapolated Observation for determination of Stomatal-Index

2.6. Palisade Ratio

The average number of palisade cells that are present beneath one epidermal cell is called as Palisade Ratio. Four continual epidermal cells are taken for the determination. It was performed by taking a leaf sample and treating it with Chloral Hydrate solution. Then the transverse-section was cut and was washed with distilled water followed by mounting it in a clean glass slide and subsequently covering it using cover slip. Using a Projection microscope, focus was adjusted till the epidermal cells and palisade cells were visible. 100x magnification was used for a clear image. Photograph was taken and then using computer graphical software, four continual epidermal cells were highlighted and then the below palisade cells were counted in that region. Palisade Ratio was calculated by the formula - Palisade Ratio = [Number of Palisade cells(32) / Number of Epidermal Cells(4)].

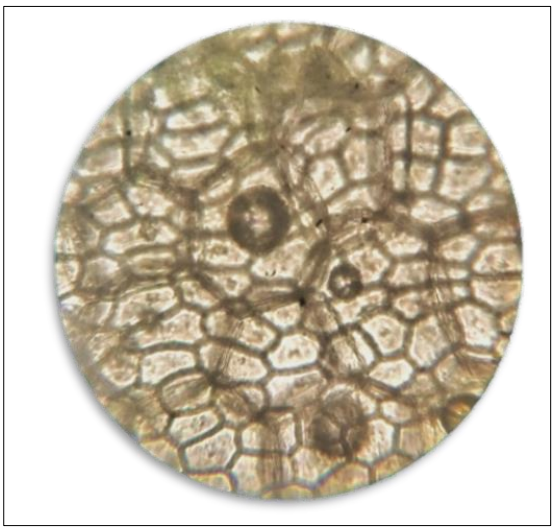

Figure 5 Microscopic Observation of Transverse Section of Leaf under 100x Magnification

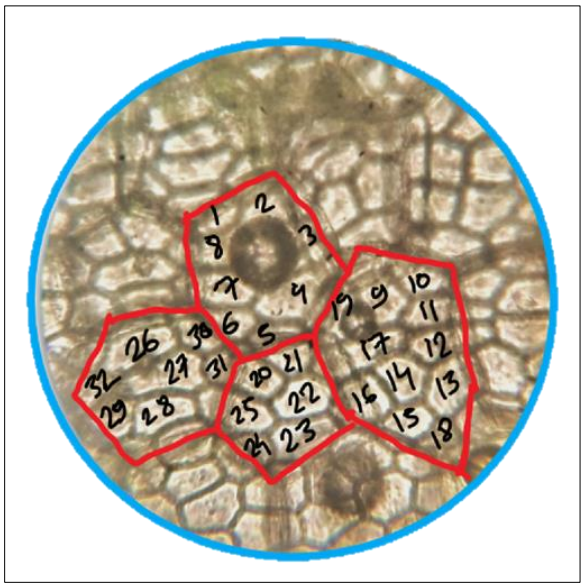

Figure 6 Extrapolated Observation for determination of Palisade Ratio

From our experimental observations, the number of Epidermal Cells considered were 4 and number of Palisade Cells found were 32. Thus, the Palisade Ratio is 8.

2.7. Vein-Islet Number

Vein-islets are the very small area of green photosynthetic tissue, encircled by the division of the conducting strands(veinlets). It can also be defined as number of vein-islets per square millimeter of the surface of the leaf. It is observed in central region of the leaf, between the mid-rib and the margins. Vein-Islet Number can be determined by taking a leaf sample and treating it with chloral hydrate solution. Then using a Camera Lucida and a black chart paper mounted on a drawing board, the stage micrometer was set and using a 16mm objective, a line was drawn using white pencil that was equivalent to be 1 mm as seen through the microscope. Then the lines were joined to construct a square. The glass slide was set which consists the cleared leaf section. The square was adjusted to be in the microscope's centre of field. The veins were traced using white pencil which are only present with in the drawn square and completing the outline of those islets those overlaps two or three of the adjacent sides. The Vein Islets are counted and numbered.

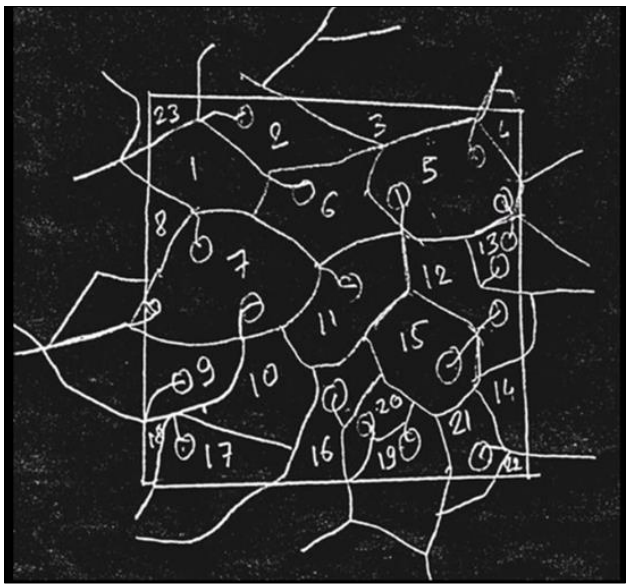

Figure 7 Vein-Islet Number

Observed count of Vein-Islets were found to be 23.

2.8. Vein-Termination Number

The number of Veinlet terminations present per square millimeter in the leaf surface in the region between the leaf margins and midrib is called Vein-Termination Number. A vein termination ultimately is the free termination of a veinlet present in a leaf. The same square that was previously used for vein islet determination was measured and the same glass slide was taken for determination of vein termination number. The vein terminations were marked with a circle. It was counted and noted.

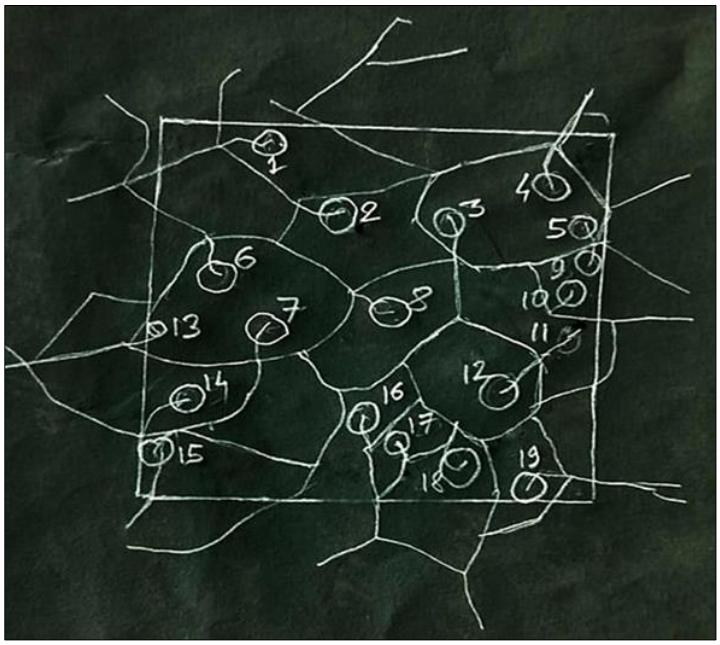

Figure 8 Vein Termination Number

The Observed Vein-Termination Number found was 19; which is characteristics to Insulin Plant.

Figure 9 Anomocytic Stomata

Figure 10 Leaf of *Costus igneus* N.E. Br

3. Results and discussion

From the set of experiments, we have found the following observations-

Table 1 Leaf Constants

Sl no.	Leaf constants	Result
1	Stomatal Number	40 ± 2
2	Stomatal Index	16.66 ± 0.02
3	Palisade Ratio	8 ± 0.02
4	Vein-Islet Number	23 ± 0.8
5	Vein Termination Number	19 ± 1

The findings following the Standardized protocols mentions that the microscopical analysis of the Insulin Plant leaves and the newly tried microscopical identical property can be properly followed for the Quality Control of this herb. The analytical numerical value seems to be characteristics of this particular phytomedical species.

The found values are characteristics to the leaf constants shows that the biostatistically conferred results may be repeated for future quality control standards.

4. Conclusion

From the experimentation, by performing Qualitative Microscopy we have calculated and estimated all the Leaf Constants of the leaves of *Costus igneus* N.E.Br. This data can be further implemented for future experimentation and Identification and for determination of Adulteration of *Costus igneus* N.E. Br. variety of India.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors express no conflicts of interest.

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