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PHARMACOGNOSTICAL CHARACTERIZATION OF *STEVIA REBAUDIANA* BERT. LEAVES: VALUABLE ANTIDIABETIC PLANT

Aisha Razi *, Sumbul Rehman and Sehrish Khan

Department of Ilmul Advia, Faculty of Unani Medicine, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh - 202002, Uttar Pradesh, India.

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Correspondence to Author:
Aisha Razi

P.G Scholar,
Department of Ilmul Advia,
Faculty of Unani Medicine, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh - 202002, Uttar Pradesh, India.

E-mail: raziaisha63@gmail.com

ABSTRACT: Globally, traditional herbal medicine has played a crucial role in primary healthcare. The recent surge in research on herbal medicine systems has led to a higher demand for medicinal plants in the global phytomedicine market. However, this increased demand also raises the risk of adulteration of raw drugs, posing potential threats to consumer health and safety. Thus, there is a pressing need for proper identification and pharmacognostical evaluation of crude medicinal plants. Addressing these concerns, a study was conducted on *Stevia rebaudiana* Bert. Linn. to assess its pharmacognostical features for accurate identification, focusing on various morphological and microscopic characteristics of the leaves. Parameters such as Stomatal index, Stomatal number, Palisade ratio, vein-islet number, and vein termination number were also analysed. This study holds significance in pharmacognostical identification, as its findings may contribute substantially to ensuring the integrity and authenticity of *Stevia rebaudiana* leaves in future.

INTRODUCTION: *Stevia rebaudiana* Bert.: *S. rebaudiana* Bert., belonging to the Asteraceae family, is native to tropical North America and South America. Its journey began in 1968 when it was exported to Japan, sparking global interest. Since then, this crop has been introduced to numerous countries, including Tanzania, Korea, Mexico, Brazil, the United States of America, Indonesia, Canada, and India [1, 2, 3, 4, 5]. Stevia leaf, also known as Sugar Leaf or Sweet leaf, holds considerable economic importance due to abundant natural sweeteners found in its leaves.

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Stevia leaf, derived from the *Stevia rebaudiana* plant, serves as a natural sweetener due to its unique composition of steviol glycosides. These glycosides, such as stevioside and rebaudioside, are non-caloric compounds, meaning they provide sweetness without contributing to caloric intake. When consumed, these glycosides interact with taste receptors on the tongue, particularly the sweet taste receptors, eliciting a sweet sensation.

Despite their sweetness, steviol glycosides are not metabolized by the body in the same way as sugar, hence they do not affect blood sugar levels or caloric intake. Moreover, stevia leaf as a natural sweetener offers advantages over artificial sweeteners. It is considered safe for consumption, with regulatory agencies such as the FDA and EFSA approving its use as a food additive. Additionally, stevia leaf is heat-stable, making it suitable for cooking and baking applications.

These natural alternatives prove beneficial for individuals with diabetes seeking alternatives to sugar.

Botanical Classification of *Stevia rebaudiana* [5]:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Group: Monochlamydae

Order: Asterales

Family: Asteraceae

Subfamily: Asteroideae

Tribe: Eupatorieae

Genus: *Stevia*

Species: *rebaudiana*

The current study aimed to assess the macroscopic and microscopic features, as well as quantitative parameters, so as to ascertain the pharmacognostic attributes of *Stevia rebaudiana* Bert. leaves.

MATERIAL AND METHODS:

Collection and Authentication of Botanical Material: Fresh leaves of *Stevia rebaudiana* Bert. were gathered from the botanical garden of Department of Botanyat Aligarh Muslim University. The leaves were identified with assistance from officials in the Botany Department and were subsequently verified in the pharmacognosy laboratory within the department of Ilmul Advia, utilizing existing literature. These leaves were identified in the pharmacognosy laboratory of Department of Ilmul Advia using available literature, and were confirmed by the lab's supervisor. To ensure future reference, a voucher number [SC-394/24] was assigned and submitted to the Ibn Baitar Museum within the Department of Ilmul Advia.

Preparation for Examination: Collected fresh leaves of *Stevia rebaudiana* Bert. were washed with water and cleared of foreign matter and other contaminants. Microscopic evaluation was done by using standard methods. Chemicals The chemicals like safranin stain, ethanol, glycerine, distilled water etc. were used. The entire chemicals, used in experiments were of analytical grade.

Macroscopic Examination: The fresh leaves of both the plants were evaluated for various organoleptic parameters such as the shape, size, colour, margin, texture, apex, presence or absence of petiole, phyllotaxy etc.

Microscopic Examination: The histological features such as number of epidermal cells, structure, distribution and type of stomata, structure and distribution of trichomes on the fresh leaves have been analyzed.

Transverse Section of the Leaves: To prepare thin transverse sections of the leaves, fully mature leaves were collected and rinsed thoroughly with water. Using a sharp blade, thin sections were carefully sliced from the central part of the leaf lamina and immersed in water to maintain moisture. Subsequently, these thin transverse sections were transferred onto a watch glass containing 2-3 drops of safranin. After allowing some time for staining, the sections were removed and washed with water. Any remaining surplus stain was removed using ethanol. Following staining, the slides were covered with a coverslip and examined under an OPTIKA binocular digital microscope B-290 at 40X magnification.

Quantitative Microscopy: The count of epidermal cells, stomata, and stomatal index of the leaves were assessed following the procedure outlined by Mohan Ram and Nayyar [6], with minor adjustments. Fresh leaves were immersed in water to remove any contaminants. After clearing the leaves, thin transverse sections were made and treated with safranin for staining. Subsequently, the sections were mounted using glycerin and examined under a compound microscope.

Epidermal Cell Number: The average number of epidermal cells per square millimetre unit of epidermis is termed as epidermal cell number.

Stomatal Number: The average number of stomata per square millimetre of epidermis is termed as stomatal number.

Stomatal Length and Osteolar Length: It was measured with the help of OPTIKA microscope software available.

Stomatal Index: The percentage of number of stomata to the total number of epidermal cells (wherein each stomata also is considered to be a single epidermal cell), is termed as stomatal index. The stomatal Index was calculated using the standard formula given below.

Stomatal Index was calculated as per following formulae [7]:

$$\text{Stomatal Index} = (S \times 100) / (S+E)$$

S = Quantity of stomata per unit area, E = number of ordinary epidermal cell in same unit area.

Vein Islet Number: it is the average number of vein islet enclosing small green in 1 mm square leaf surface.

Vein Termination Number: Vein termination number is the average number of veinlet terminations per mm of leaf surface taken from region of midrib to margin of the leaf.

Procedure: To determine the vein islet and vein termination numbers, sections of the leaf lamina situated between the midrib and margin were cut into small pieces measuring 1-3 mm square. These pieces were then boiled in a concentrated solution of chloral hydrate for approximately 15 minutes until discoloured. Following this, the transparent fragments were placed onto a glass slide and observed under a microscope at 10X magnification. The count of vein islets was conducted within a 1 mm sq. area, while vein terminations were similarly counted within 1 sq. mm area. To ensure accuracy, three readings were taken for each vein islet and vein termination number, and the slides were photographed.

Palisade Ratio: palisade ratio is defined as the average of palisade cells below single epidermal cell [8].

Procedure: For this study, small sections (1-2mm) of leaves cultivated under full sunlight were selected. These sections were cleared by boiling them in a 200% chloral hydrate solution. Subsequently, the cleared sections were mounted and observed under a microscope. Initially, the focus was set on groups consisting of four epidermal cells. Following this, a slight adjustment of the fine focus allowed for the underlying palisade cells to be brought into focus within the same area covered by the four epidermal cells. The palisade ratio was then calculated by dividing the number of palisade cells observed by 4. To ensure accuracy, five readings were taken from different sections to obtain a reliable average value.

RESULTS AND DISCUSSION

Macromorphology: The results of macroscopic and organoleptic examination revealed that *Stevia rebaudiana* Bert. is an upright, perennial herbaceous species that grows up to 90 cm tall. The plant has tap roots. It has small, simple, sessile, oval-elliptical in shape leaves, that are 3 to 5 cm long and 1 to 1.5 cm wide, arranged in an opposite decussate pattern (two successive pairs of leaves occur perpendicular to each other). The margin is serrate with reticulate venation. The flowers bear racemose type inflorescence **Fig. 1A, B, C, D** and **Table 1.**

Microscopic Analysis: The trans-sectional view (T.S.) of *S. rebaudiana* leaves exhibit several distinct features including the epidermis, endodermis, palisade parenchyma, and vascular bundles. When the leaves are sectioned from the midrib, they reveal specific characteristics. Notably, two types of trichomes are evident: glandular capitate type and nonglandular uniseriate multicellular type consisting of 6-8 cells. Additionally, the cross-section displays stomata of Anomocytic or Ranunculaceous type on the abaxial surface, with fewer occurrences observed on the adaxial surface. The mesophyll consists of both palisade and spongy parenchyma layers. **Fig. 2A, B, C, D**).

Quantitative Microscopy: The *Stevia rebaudiana* Bert. leaf exhibited a wide range of characteristics: the epidermis comprised 100-110 cells, with 35-40 stomata present. The stomatal index on the abaxial surface ranged from 25.92 to 27.58. Stomatal length measured 0.13µm, while osteolar length was 0.07µm. Vein islet number varied between 4-7, and vein termination number ranged from 3-5. Additionally, the palisade ratio was recorded as 2.5-3.5 (refer to **Table 2**).

LEAF CONSTANTS

Stomatal No	35-40
Stomatal Index	25.92-27.58
Palisade Ratio	2.5-3.5

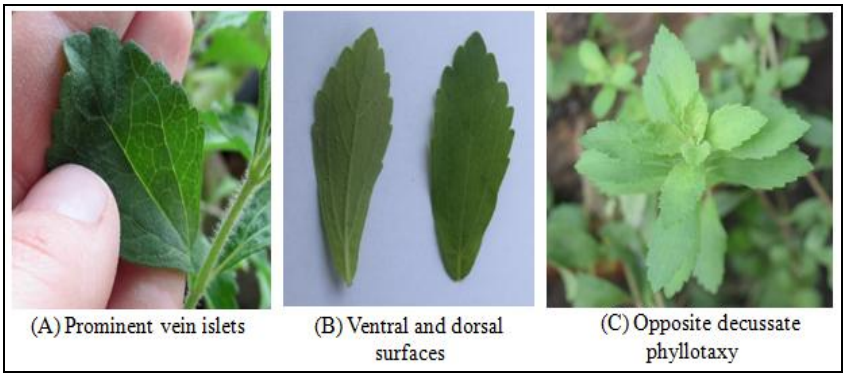

FIG. 1: *STEVIA REBAUDIANA* BERT. MORPHOLOGICAL CHARACTERS OF LEAF

TABLE 1: ORGANOLEPTIC FEATURES OF LEAVES OF *STEVIA REBAUDIANA* BERT

Parameters	Leaves of *Stevia rebaudiana* Bert.
Leaf type	Simple
Phyllotaxy	Opposite
Petiole	Sessile
Stipule	Absent
Shape	Oval-elliptical
Size (l*b) cm	3-5/1-1.5
Venation	Reticulate
Apex	Obtuse
base	Attenuate
Margin	Serrate
Glands at leaf base	Absent
Trichomes	Glandular capitate type and no glandular uniseriate multicellular
Surface appearance	Glabrous on upper surface and trichomes present on lower surface
Odour	Odourless
Taste	Sweet

TABLE 2: QUANTITATIVE PARAMETERS OF LEAVES OF *STEVIA REBAUDIANA* BERT

Parameters	*Stevia rebaudiana* Bert.
Stomata No.	35-40
Epidermal Cell No.	100-110
Stomatal Index	25.92-27.58
Vein Islet Number	4-7
Vein Termination Number	3-5

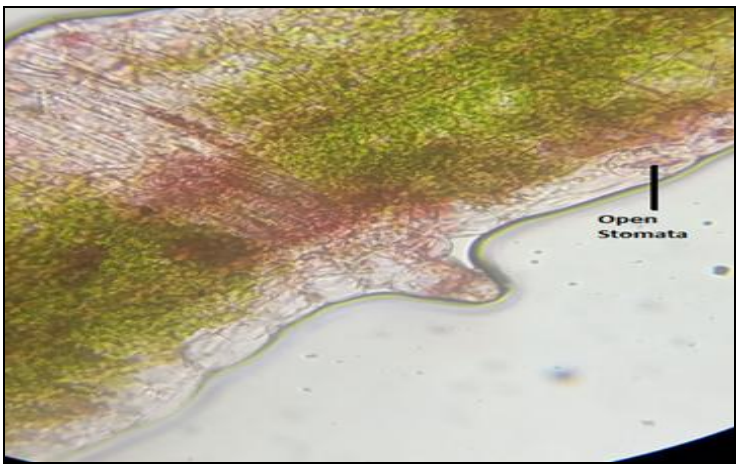

FIG. 2A: T.S. OF *STEVIA REBAUADIANA* SHOWING STOMATA ON ABAXIAL SURFACE

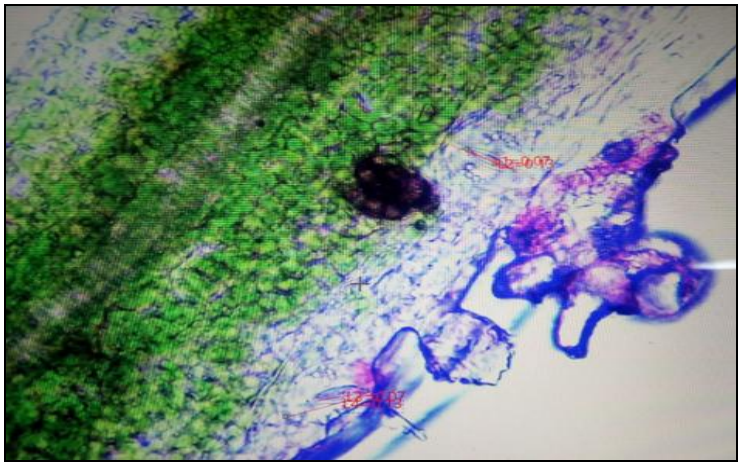

FIG. 2B: L1 OSTEOLAR LENGTH, L2 STOMATAL LENGTH OF *STEVIA REBAUDIANA*

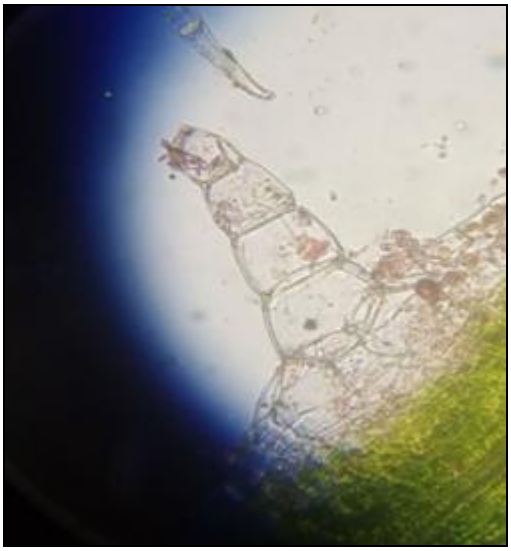

FIG. 2C: T.S. SHOWING NONGLANDULAR UNISERIATE MULTICELLULAR TRICHOME

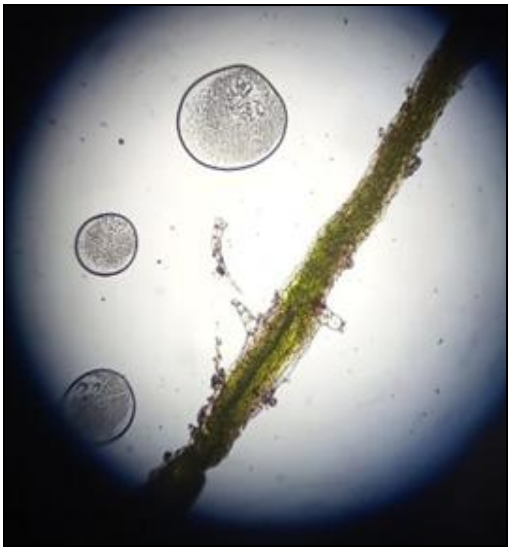

FIG. 2D: T.S. SHOWING GLANDULAR TRICHOME IN *S. REBAUDIANA* LEAF

CONCLUSION: This research aims to establish diverse pharmacognostic criteria for *Stevia rebaudiana* Bert. leaves. The leaves possess a sweet taste, serrated margins, and an oval-elliptical shape. Microscopic examination reveals distinctive features including the absence of glandular trichomes, presence of glandular trichomes, and ranunculaceous stomata.

The parameters investigated in this analysis will not only aid in quality assessment but also in the identification of adulterants in herbal drugs, or they can serve as key components in herbal medicine formulations.

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CONFLICTS OF INTEREST: All authors declare there are no conflicts of interest related to this study.

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