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PHARMACOGNOSTIC CHARACTERIZATION OF THE LEAVES OF *STEVIA REBAUDIANA* BERT

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ABSTRACT: For the last few centuries, the leaves of *Stevia rebaudiana* have been used as an efficient substitute for sugar due to its sweetness and almost negligible Glycemic effects. The study concentrates on the pharmacognostic aspects and other anatomical characteristics of the leaves. The study is performed on the plant leaves collected from the medicinal plant garden of Birla Institute of Technology, Mesra, Ranchi, India. The study is purely concentrated on finding various morphological characteristics and microscopic characteristics of the leaves. The microscopic characterization includes understanding the various anatomical characters of the leaves as well as the identification of the various diagnostic characteristics related to the leaves. Leaf constants, such as the Stomatal index, Stomatal number, Palisade ratio, vein-islet, and vein termination numbers, were also determined. The study also aims to determine various physicochemical parameters such as Moisture content, total ash, and extractive values. Preliminary phytochemical characterization of the aqueous extract of the leaves was also performed.

INTRODUCTION: *Stevia rebaudiana* Bert. is a perennial shrub of Asteraceae family, native to tropical and subtropical regions of North America and South America. It is commonly known as Sugar leaf, Sweetleaf or simply Stevia. Apart from these regions, the plant is also extensively cultivated in countries like Japan and India. The plant, though in use for the last few hundred years by the natives of Peru, has gained immense recognition recently due to its use as a natural sweetener.

The leaves of the plant have been used due to its almost negligible diabetic effect. The leaves contain a class of diterpene glycosides known as Steviol glycosides. The major component of the Steviol glycoside is known as Stevioside, which is an ent-kaurene type of Diterpene [1].

The genus *Stevia* consists of 240 species of plants native to South America, Central America, and Mexico, with several species found as far north as Arizona, New Mexico, and Texas. They were first researched by Spanish botanist and physician Petrus Jacobus Stevus, from whose surname originates the Latinized word *Stevia*. The leaves of *Stevia* are 30-45 times sweeter than sucrose. The leaves can be eaten fresh or put into various health drinks or foods. The plant was used extensively by Guarani people for more than 1500 years, and the plant has a long history of medicinal use in

Paraguay and Brazil to sweeten local teas, medicines, and a "sweet treat".

Stevia can be used as an anti-obesity, anti-diabetic, antihypertensive drug, and also in heartburn. It is also proved helpful in lowering uric acid levels, preventing pregnancy, and increasing muscle contraction strength that pumps blood from heart.

In foods, *Stevia* is used as a non-caloric sweetener and flavor enhancer. *Stevia* was originally available as a "dietary supplement" in the U.S. It was not allowed as a "food additive" until 2008. *Stevia* is also available as a sweetener in countries like Japan, South Korea, Malaysia, Taiwan, Russia, Israel, Mexico, Paraguay, Uruguay, Venezuela, Brazil, Colombia, and Argentina [2, 3].

MATERIALS AND METHODS:

Collection and Authentication of the Plant: The plants were collected from the medicinal plant garden of Birla Institute of Technology, Mesra, Ranchi, Jharkhand, from July to November 2013. The plant was authenticated by Central National Herbarium, Botanical Survey of India, Kolkata, West Bengal. A specimen voucher has been deposited (CNH/23/2014/TechII/63). The plant was also identified by Prof. (Dr.) S. Jha, Dept. of Pharmaceutical Sciences, BIT, Mesra.

Macroscopic Analysis: Thorough morphological and organoleptic characterizations of the leaves were performed. Characters like size, shape, colour, venation, margin, apex, texture, lamina *etc*. were recorded in accordance to standard textbooks [4, 5].

Transverse Section of the Leaves: [6, 7] For preparing thin transverse sections of the leaves, mature leaves were collected and washed with water. Thin sections of the leaves were cut from the middle portion of the lamina and kept in water, so that they retain moisture. Staining agents like Safranin, Phloroglucinol, and dil. HCl were used. Microphotographs were taken using Leica DME, DSC Power shot S70 DE [PICT Bridge], 7.1 megapixels, wide-angle 3.6X optical zoom lens.

Powder Microscopy [8, 9]: Leaves of the plant were dried in the shade and passed through a sieve no. 180 and a sieve no. 125, separately, to obtain fine powder and then subjected for powder microscopy. A judicious amount of the powder was taken on a

glass slide, and a few drops of chloral hydrate were added. The slide was then heated for a few seconds carefully to avoid drying of chloral hydrate. A coverslip was placed over the slide carefully to avoid air bubbles. An extra amount of chloral hydrate was removed using blotting paper. Lignified tissues were confirmed by staining with phloroglucinol and conc. HCl. On a different slide, a mixture of phloroglucinol and conc. HCl in the ratio 1:1 was added and observed under a microscope.

Leaf Constants: Fresh leaves were collected, washed and small rectangular portions were cut from the middle region of the lamina. The small cut pieces were put in a test tube, and a small amount of Choral hydrate was added. Then the test tube was heated so that the components of it are boiled. The treatment with Choral hydrate helps in removing the chlorophyll.

Stomatal index, Stomatal number, Vein islet number, Vein termination number, and palisade ratio were recorded by using camera lucida, stage micrometer an ocular micrometer.

Physicochemical Parameters: Physicochemical parameters such as ash value, water-soluble ash value, acid insoluble ash value, moisture content (loss on drying), and extractive value were performed. The leaves were collected and air-dried. The parameters were checked according to the official methods [10].

RESULTS AND DISCUSSION:

Macroscopic and Organoleptic Parameters: The detailed morphological and other organoleptic parameters are reported in **Table 1**.

TABLE 1: MORPHOLOGICAL CHARACTERS OF LEAVES OF *STEVIA REBAUDIANA*

Morphological and Organoleptic parameters	Characters
Taste	Sweet
Odour	Characteristic
Colour	Green
Texture	Glandular pubescent upper layer
Size	2-3 cm
Shape	Oval-elliptical
Margin	Serrate
Venation	Reticulate
Apex	Obtuse to Subacute

The leaves are arranged in an opposite decussate pattern. The leaves are 2-3 cm in length and are oval-elliptical in shape. The margin is serrate with reticulate venation.

Microscopic Analysis:

Anatomy: Anatomical study was done for the transverse section (T.S.) of the leaves of the plant *Stevia rebaudiana*. T.S. of the leaf showed the presence of epidermis, endodermis, palisade parenchyma, vascular bundles *etc*. A detailed characterization of the transverse section of the leaves of the plant has been reported in **Fig. 1** and **Fig. 2**.

The transverse section of the leaves is cut from the midrib, and it has shown various characteristics.

There are two types of trichomes present in the leaves, glandular and tector. The transverse section also reveals the presence of two different types of tector trichomes. One of them is slightly longer with the elongated ending and is 8-10 celled, while the other one is relatively shorter and thinner. The longer trichome seems to be conical uniseriate, while the shorter one is thinner and is comprised of 3-6 cells. The transverse section also reveals anomocytic or Ranunculaceous or irregular called stomata at the abaxial surface, while occurrences of the stomata are scarce in the adaxial surface. Both upper and lower epidermis is thinner, and the mesophyll is comprised of palisade and spongy parenchyma.
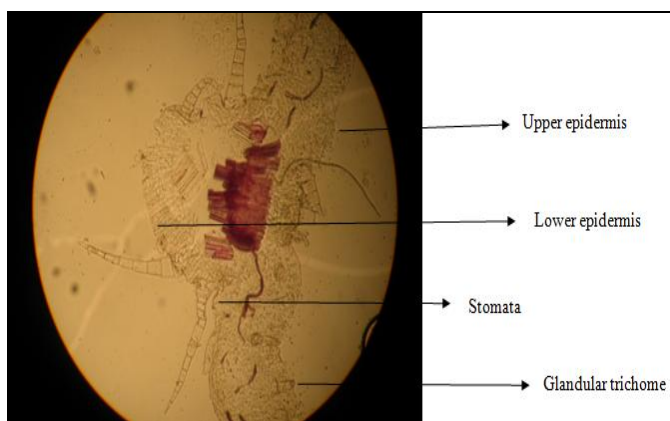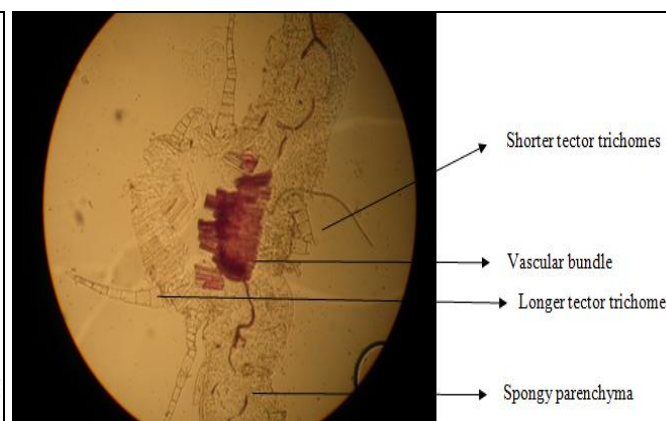

FIG. 1: TS OF THE LEAVES OF *STEVIA REBAUDIANA*

FIG. 2: TS OF THE LEAVES OF *STEVIA REBAUDIANA*

Powder Microscopy: Powder microscopy of the leaves of *Stevia rebaudiana* has shown various diagnostic characteristics of leaves. The microscopy has revealed the presence of glandular trichomes, tector trichomes, hair on the epidermal surface, xylem vessels with annular, scalariform, and spiral thickening. There are two types of non-glandular or tector trichomes present in the epidermal layer. The longer tector trichomes are conical, erect, uniseriate, and are composed of 8 to 10 cells, whereas the shorter ones are thinner, uniseriate, and are composed of 3 to 6 cells. The characteristics of the leaves have been reported in **Fig. 3**, **Fig. 4,** and **Fig. 5**.
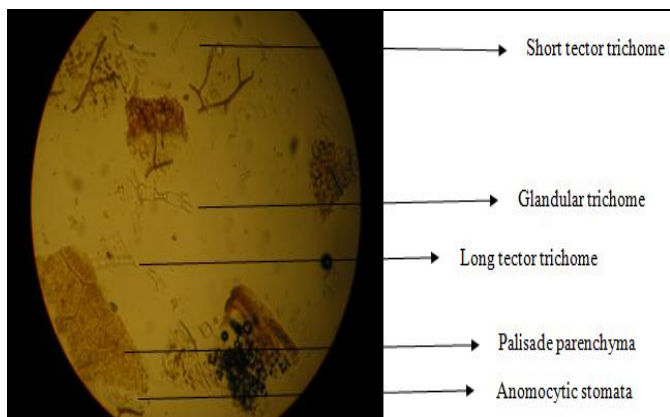

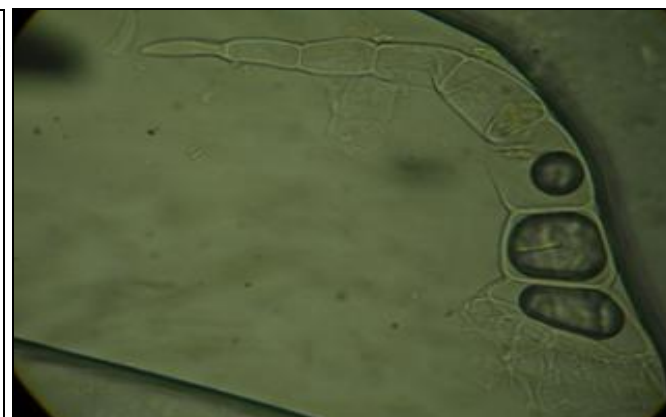**FIG. 3: POWDER MICROSCOPY OF THE LEAVES OF *STEVIA REBAUDIANA***

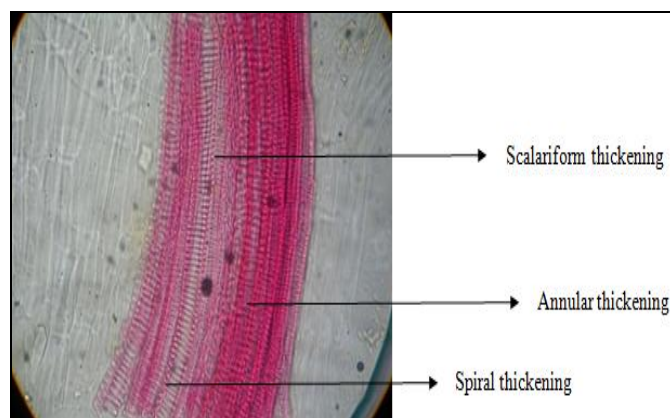**FIG. 4: LONG CONICAL, ERECT, UNISERIATE TECTOR TRICHOME**

FIG. 5: XYLEM VESSEL WITH ANNULAR, SPIRAL AND SCALARIFORM THICKENING, STAINED WITH PHLOROGLUCINOL AND HCl

Leaf Constants: Leaf constants are important parameters for the quantitative analysis of a leaf. They include parameters like the Stomatal index, Stomatal number, Palisade ratio, Vein islet, and Vein termination number. These parameters help in quantification of crude drugs when they are leaves. The findings are reported in the following table. Vein islet number of the leaves was found to be between 4-7, and the Vein termination number was reported to be between 3-7.

TABLE 2: LEAF CONSTANTS FOR THE LEAVES OF *STEVIA REBAUDIANA*

Leaf Constants	Stomatal Index	Stomatal Number	Palisade Ratio
Upper Epidermis	7.14- 14.28	29-43	3.2-4.8
Lower Epidermis	22.22-27.27	93-118	2.5-3.5

TABLE 3: PHYSICOCHEMICAL CHARACTERS OF THE LEAVES OF *STEVIA REBAUDIANA*

Physicochemical Parameters	Value determined in % (w/w)
Moisture content	7.13
Total ash	9.31
Acid insoluble ash	1.94
Water-soluble ash	4.82
Water-soluble extractive value	36
Alcohol soluble extractive value	34.8

Physicochemical Analysis of the Leaves: Physicochemical parameters such as moisture content (Loss on drying), Total ash value, acid insoluble ash value, water-soluble ash value and extractive value were determined. Ash value of a crude drug helps in determining the impurities such as earthy contaminants of a drug. The moisture content of a drug is a measurement of the amount of free water present in a drug, whereas the extractive value of a drug is useful in finding adulterants present in the crude drug. The findings are reported in the following table.

CONCLUSION: This study is carried out to lay down various pharmacognostic standards for the leaves of *Stevia rebaudiana*. There is no thorough pharmacognostic record of the leaves of such an important plant and this study was done in hope to be able to lay down few standards. The leaves have shown to be sweet in taste, have serrate margin and oval-elliptical shape. The microscopy of the leaves has shown various diagnostic characters like tector trichomes, glandular trichomes, and Ranunculaceous stomata. The powder microscopy has revealed two types of tector trichomes and xylem vessels with different thickening. This study is in the hope of laying a foundation to further studies regarding its complete chemical profiling.

DECLARATION OF INTEREST: The authors declare no conflict of interest.

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