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Pharmacognostic and Phytochemical Investigation of *Stevia rebaudiana*

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ABSTRACT

Stevia rebaudiana is the single sweetener which has antidiabetic property. The plant is also used for treatment of a number of ailments like hypertension and hyperlipidemia. The current study was therefore carried out to provide requisite pharmacognostic details about the plant. Pharmacognostic investigation of the fresh, powdered and anatomical sections of the leaves of *Stevia rebaudiana* was carried out to determine its morphological, anatomical, and phytochemical diagnostic features. Quantitative diagnostic characteristics, physicochemical properties and qualitative and quantitative phytochemical measures were established. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

KEY WORDS: *Stevia rebaudiana*, Pharmacognostic standardization, Physicochemical analysis

INTRODUCTION

The herbal drug industry is considered to be a high growth industry of the late 90s and seeing the growing demand, it is all set to flourish in the next century. The trend for the increasing popularity of medicinal herbs in countries like America, Australia and Germany is well supported by statistical data. Ayurveda, the ancient Indian system of medicine, strongly believes in polyherbal formulations and scientists of modern era often ask for scientific validation of herbal remedies.

The efficacy of some herbal products is beyond doubt, the most recent examples being *Artemisia annua* (artemesinin), *Taxus brevifolia* (taxols) and *Silybium marianum* (Silymarin). *Hypericum perforatum* (hypericin & hyperforin), *Allium sativum* (allicin or allin), *Ginkgo biloba* (Ginkgolic acid) are popularly used herbal remedies among people. All these herbals are standardized for active constituent. Standardization means adjusting the herbal drug preparation to a defined content of the active constituent. Extract refers to a concentrated preparation of active constituent of a medicinal herb. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals.

Medicinal plant materials are characterized according sensory microscopic and macroscopic characteristics. Taking into consideration the variation in sources of crude drugs and their chemical nature, they are standardized by using different techniques including the method of estimation 0f chief active constituent. Organoleptic evaluations can be done by means of organs of sense. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample.

A number of different bases are used for morphological studies and natural variations in these characteristics plays an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as for as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs. This study is an attempt to establish the standardization parameters for complete pharmacognostic evaluation of stevia.(1)

Stevia, botanically known as *S. rebaudiana,* originally came from rain forests of Brazil and Paraguay. Now it is also cultivated in Japan, Korea, Thailand, China and India. About 200 species are native to South America and it is also found in Israel and Central America. Stevia has been used to sweeten tea for centuries, dating back to the Guarani Indians of South America. For hundreds of years, native Brazilians and Paraguayans have employed the leaves as a sweetening agent. Europeans learned about the plant in 16th

century, whereas North American interest began to 20th century when researchers heard of its sweetening property. Paraguayan chemist Ovidio Rebaudi documented stevia in early 1900's then in 1905 a botanist of same country Moises Bertoni gave the present name to the plant. Glycoside responsible for sweetening were discovered in 1931. Stevia extracts are used today as food additives by the Japanese and Brazilians as a non calorie sweetner.

Over 100 phytochemicals have been discovered in stevia since. It is rich in terpenes and flavonoids. Stevia contains a complex mixture of diterpenes, triterpenes, stigmasterol, tannins and volatile oil. The constituents responsible for stevia's sweetness were documented in 1931, when eight novel plant chemicals called diterpenic glycosides (5-14%) were discovered and named as Stevioside, Dulcoside and Rebaudioside A, B, C, D and E. (2, 3, 4 , 5) (Fig.1)

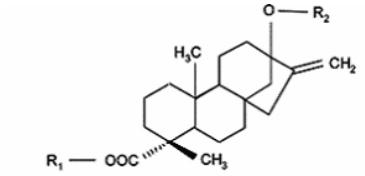

Figure 1 : Common structure of stevia glycosides

The main plant chemicals in stevia include: apigenin, austroinulin, avicularin, beta-sitosterol, caffeic acid, campesterol,caryophyllene, centaureidin, chlorogenic-acid,chlorophyll, cosmosiin, cynaroside, daucosterol, diterpene glycosides, dulcosides A-B, foeniculin, formic acid, gibberellic acid, gibberellin, indole- 3-acetonitrile,isoquercitrin,isosteviol, jhanol, kaempferol, kaurene, lupeol, luteolin, polystachoside, quercetin, quercitrin, rebaudioside A-F, scopoletin, sterebin A-H, steviol, steviolbioside, steviolmonoside, stevioside, stevioside a-3, stigmasterol, umbelliferone, and xanthophylls.

Stevioside

- Stable at high (100^0C) temperature
- Stable at a range of pH values (3-9)
- Non Calorific
- Non Fermentable
- Not darkens upon cooking
- Approximately 250 times more sweet than cane sugar(6).

Stevia has been used for centuries as a natural sweetner and may be helpful in treating diabetes. Plant effects on blood pressure, act as a vasodilator in both normal and hypertensive animals. It has also produced decrease in B.P. and has increased diuretic and netriuretic effects in rats. The plant has cardio tonic action which normalize B.P. and regulates heart beat.Stevia extract has exhibited strong bactericidal activity against a wide range of pathogenic bacteria including certain E. coli strains. Steviol, stevia's aglycone is mutagenic towards Salmonella and other bacterial strains under various conditions and towards certain cell lines. It may also be effective against Candido albicans. It may also play a role against dental plaque. The drug is neither mutagenic nor genotoxic and chronic administration to male rats had no effect on fertility vs control. It is used as a sweetening agent, hypotensive, hypoglycemic and bactericidal(7,8).

MATERIALS AND METHODS

Collection

Leaves of *S. rebaudiana* were collected in the month of April from Misrod, Bhopal (M.P.). Plant material was dried under shed, packed in a paper bag and stored at ambient temperature until use.

Authentication

A herbarium was prepared, authentified and deposited at Dept. of Pharmacy, B.U. Bhopal.

Morphological Properties(1,9).

The leaves of the taxon are bright green in colour having distinct, characteristic odour and sweet taste with slight bitter after taste. The leaves are about 4- 5 cm.X 1- 4 cm in size. The leaves are cuneate in shape with glabrous surface. Mature leaves having crenate margin and reticulate venation. Apex of leaves is acute with decurrent base. Presence of two secondary ribs on either sides of midrib is the main distinguishing characteristic.

Anatomical Properties

Stem of the plant showed general characteristics of a dicot stem such as, a very large pith and vascular bundles arranged in a ring. A thin section of leaf was stained with phloroglucinol and con. HCl and mounted in glycerine. Lignified cells turn red. Single layer of rectangular upper and lower epidermal cells covered with thin cuticle is clearly visible. Isobilateral, mesophyll is not differentiated into palisade and spongy parenchyma but mostly consists of only thin walled spongy parenchyma. On both the upper and lower surfaces covering, multicellular, uniseriate trichomes with pointed tip were observed. Abaxial and adaxial epidermis have anomocytic stomata. Scalariform xylem vessels turned red with phloroglucinol- HCl and present on both sides of midrib. Vascular bundles are conjoint, colleteral and lignified. Few lignified scalernchymatous cells above vascular bundles are distinguishing characteristics of the leaf. There was no starch grain seen while cystolyths was scattered in midrib region. The results

of powder analysis also supports the findings of microscopic studies.

Quantitative Microscopy

Quantitative microscopy was performed using standard procedures. (Table: 1)

Physicochemical Analysis

EXTRACTION(10) [10]

Hot water extract or decoction of leaves was prepared as described in Ayurvedic Pharmacopoeia, evaporated and dried to a powder. Dried extract was kept in a dessicator with calcium carbonate to prevent moisture absorption, because prepared extract is hygroscopic in nature and absorb moisture readily.

Preliminary Phytochemical and Physicochemical analysis(11)

The extract was subjected to preliminary phytochemical and physicochemical testing for the detection of major chemical group. (Table: 2 and Table: 3)

Phytochemical Evaluation by Thin-Layer Chromatography(12,13,14)

Absorbent: Silica Gel GF

Solvent System:

Chloroform : Methanol : Water [85 : 25 : 4]

Visualization: UV Chamber and Iodine Vapours.

One dimensional Thin Layer Chromatography was performed with and with-out chamber saturation. (Table: 4)

RESULTS AND DISCUSSION

The detailed and systematic pharmacognostical evaluation would give valuable information for the future studies. The plant (*S. rebaudiana*) showed the general characteristics of a dicot plant. The microscopical study of stem showed a very large pith and vascular bundles arranged in a ring. A thin section of leaf showed the presence of non- lignified, multi-cellular, uniseriate covering trichomes on both the surfaces. The presence of anomocytic type of stomata on both the surfaces was observed but these are not very clear in 10X magnification. Single layer of rectangular epidermal cells covered with thin cuticle is observed. Isobilateral mesophyll is not differentiated into palisade and spongy parenchyma, but, mostly consists of spongy parenchyma which is thin walled and loosely arranged. In the midrib region there are conjoint, colleteral, lignified vascular bundles. Few lignified sclarenchymatous cells above vascular bundles are also visible. Starch grains are absent while cystoliths of various sizes are scattered. The powder microscopical study of leaf supported the findings of microscopical evaluation.

In quantitative microscopy, the stomatal index for upper and lower surfaces were found to be 5.8 to 7.0 to 7.6 and 8.7 to 10.3 to 11.0 respectively. Vein islet number and vein- let termination number are 8 to 13 (Average 10. 5) and 10 to 16 (Average 13) respectively. The total moisture is found to be 6.7 % along with total ash 12. 21%, of which, 3. 7 % is the acid insoluble ash. The extractive values were found to be 22. 99 %, 18. 83 % and 5.04 % dry wt. for water, alcohol and petroleum ether respectively.

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The vein islet, and vein termination numbers and the other parameters determined in the quantitative microscopy, are relatively constant for plants and can be used to differentiate closely related species. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica(15).One dimensional thin layer chromatography was performed first with pure solvents like methanol and chloroform, which gave no separation and then standard solvent system chloroform: methanol: water (65: 25: 4) was used,[14] but, no separation was observed and whole of the extract run up- to the solvent front with tailing. To improve separation the polarity of solvent system was decreased by increasing the ratio of chloroform and the chromatogram with this ratio (85: 25: 4) showed three spots at Rf 0.15, 0.21 and 0.5, which may be of rebaudioside, stevioside and other polar diterpene glycosides respectively, while chlorophyll moved up- to the solvent front. Thin layer chromatogram matches with the standard.

Since the plant, *S. rebaudiana* is the single sweetner which is also used for the treatment of diabetes, it is important to standardize it for use as a drug. The pharmacognostic constants for the leaves of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

Table 1 : *Quantitative microscopy leaves of S. rebaudiana.*

Parameter		Range
Stomatal index	Upper epidermis	5.8 to 7.0 to 7.6
	Lower epidermis	8.7 to 10.3 to 11.0
Vein islet number		8 to 13 (Avg.10.5)
Veinlet termination number		10 to 16 (Avg.13.0)

Table 2 : *Preliminary phytochemical analysis of leaves of S. rebaudiana*

Compound	Detection Test
Phenols	- ve
Tannins	- ve
Terpenoids	+ ve
Alkaloids	- ve
Anthraquinones	- ve
Flavonoids	+ ve
Carbohydrates	+ ve
Glycosides	+ ve

Table 3 : *Physicochemical analysis of leaves of S. rebaudiana.*

Parameter	Results
Moisture Content	6.7 %
Total Ash	12.21 % dry wt.
Acid Insoluble Ash	3.7 % total ash
Water Soluble Extractive	22.99 % dry wt.
Alcohol Soluble Extractive	18.83 % dry wt.
Petroleum Ether Soluble Extractive	5.04 % dry wt.

Table 4 : *Interpretation of chromatogram of S. rebaudiana leaf extract.*

Solvent System	RF Value
Methanol	No seperation
Chloroform	No separation
Chloroform: Methanol: Water [65: 25: 4]	Tailing
Chloroform: Methanol: Water [85: 25: 4]	Three spots with slight tailing 1st spot: 0.15 2nd spot: 0.21 3rd spot: 0.5
Chlorophyll moved up- to the solvent front.	

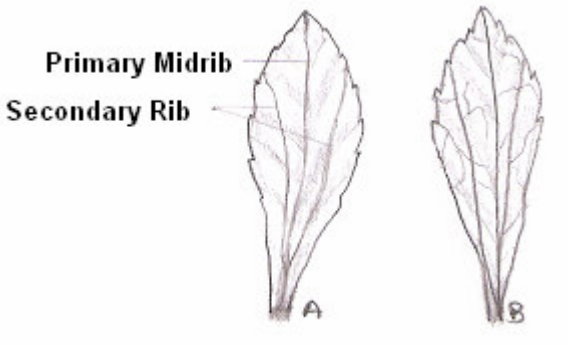

Figure 1: Leaf, A: Dorsal Surface, B: Ventral Surface.

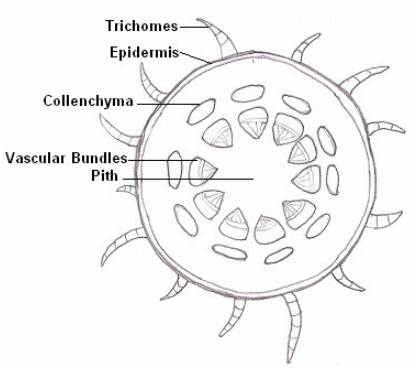

Figure 2: Schematic Diagram, T.S of stem.

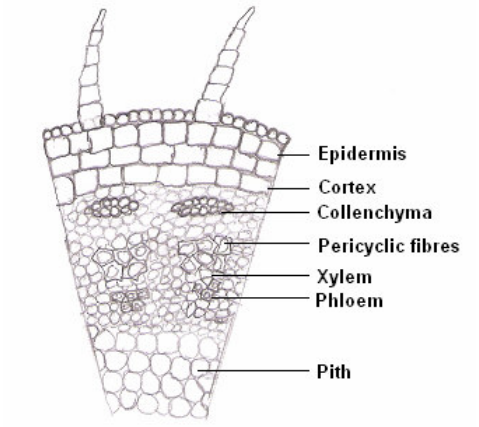

Figure 3: Cellular Structure, T.S. of stem

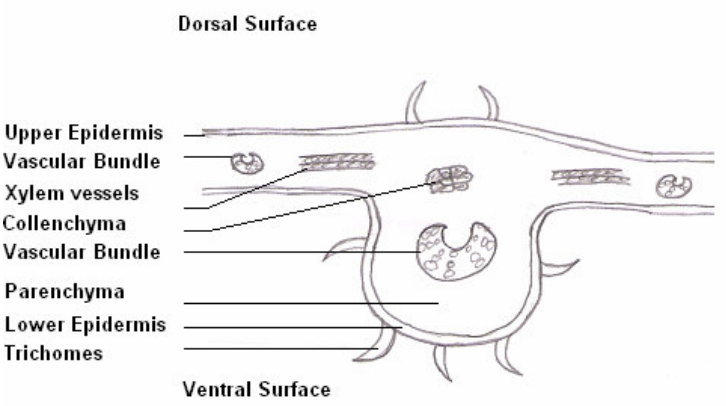

Figure 4: Schematic Diagram, T. S. of leaf.

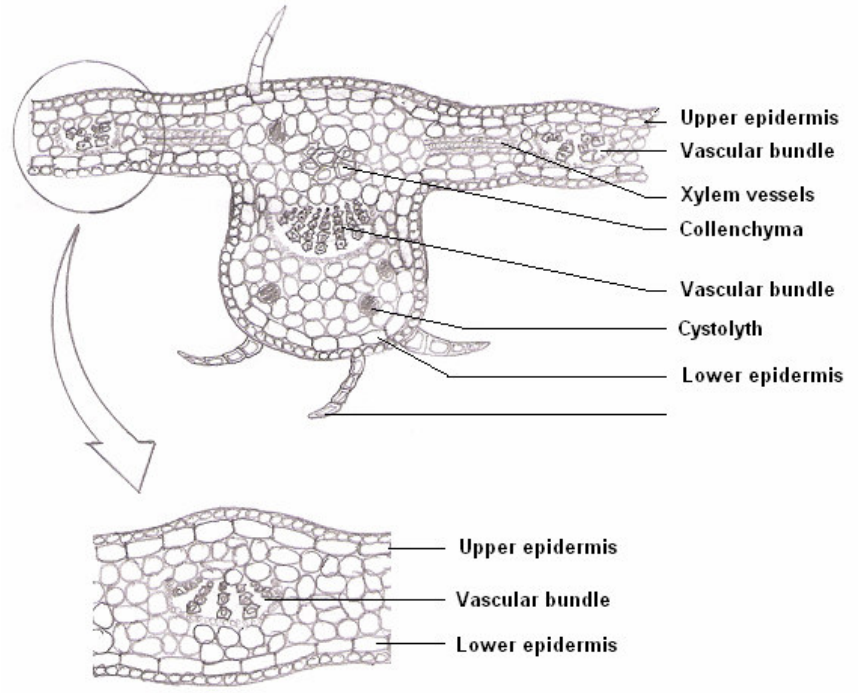

Figure 5: Cellular Diagram, T. S. of leaf

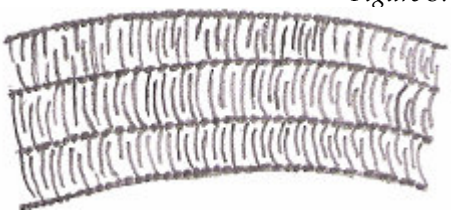

Figure 6: Xylem Vessel, scalariform.

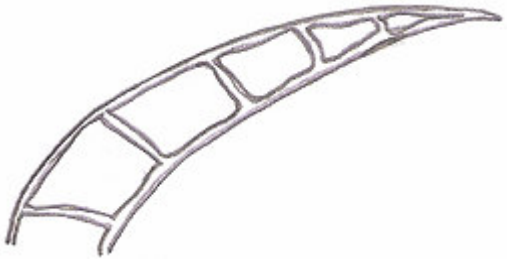

Figure 7: Trichomes, covering, multi- cellular

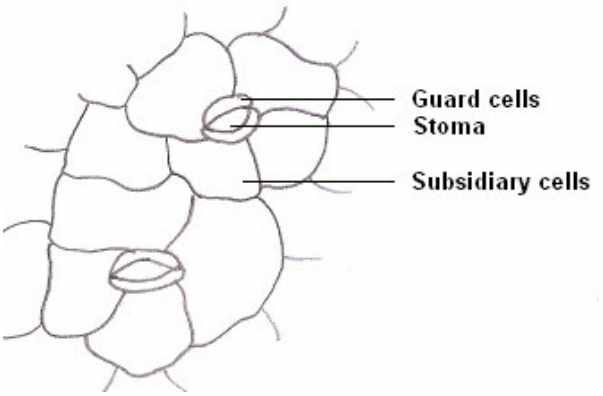

Figure 8: Stomata, anomocytic.

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