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Preliminary phytochemical and pharmacognostic studies on *Stevia rebaudiana* bertoni

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Abstract
The medicinal plant Stevia rebaudiana belongs to the family Asteraceae, tribe Eupatorieae. *Stevia rabuadiana* is originally a South American wild plant (Ejaz *et al*., 2013). The herb *S. rebaudiana* is a calorie-free sweetener hence, a better dietary supplement. Further, both *in vitro* and *in vivo* studies revealed that these glycosides possess pharmacological and therapeutic properties, including antioxidant, and anti-diabetic activity (Kajur *et al*., 2010). Keeping its medicinal significance in a view the present study is focused. The results show the presence of secondary metabolites such as phenols, Flavonoids, Triterpenes, Lactones, Tannins, Saponins, Alkaloids which in turn may be the responsible for its medicinal efficacy.

Keywords: anti-diabetic medicinal plant phytochemistry pharmacognosy stevia rebaudiana

Introduction
Stevia rebaudiana is generally recognized as candy leaf, sweet leaf, sweet leaf, or sugar leaf. It is a tender perennial native to parts of Brazil and Paraguay that favors humid, wet environments, although the root does not endure standing water. Stevia is widely grown and known for its sweet leaves. *Stevia* is sold under various trade names. The chemical compounds that produce its sweetness are various steviol glycosides (mainly stevioside and rebaudioside), which have 250–300 times the sweetness of sugar. The leaves can be eaten fresh or put in teas and foods. *Stevia* is also used as medicinal for treating heart burn and other ailments.It is recommended in treating obesity, high blood pressure and dental caries (Fajita & Edahio, 1979) [3]. Moreover, not only it has negligible effecton blood glucose but it also enhances glucose tolerance (Curi *et al.*, 1986) [2].

Materials and Methods
The leaves of *Stevia rebaudiana* was collected and shade dried, identified and authenticated using Gamble and is deposited in raw drug repository of VVPL, powdered and extracted using water by reflux method.

A. Preliminary phytochemical tests
The qualitative phytochemical tests were carried out for phenols, flavonoids, steroids, triterenes, diterpenes, lactones, tannins, lignins, saponins, alkoloids following the methods of Gibbs (1974) [1], Kleipool (1952), Peach and Tracey (1959) [6].

B. Physico chemical and Fluorescence Studies
Organoleptic characters
The present investigation comprises studies on both physical and sensory characteristics such as colour, sensation, taste, oily stain and mucilage of the species under study.

Loss on drying
Two grams of powdered drug was incinerated in a sintered silica crucible and dry the material at 60°C for 1 hour. Weighed using Anamed Electronic balance, India and not down the readings and loss on drying was calculated using the formula.

$$\textbf{\textit{Loss on drying}} = \frac{\boldsymbol{B-C}}{\boldsymbol{A}} \times \mathbf{100}$$

Where, weight of empty crucible = C, weight of plant material = A, weight of crucible after drying = B, weight of ash = B-C.

Determination of total ash
Two grams of powdered drug was incinerated in a sintered silica crucible by gradually increasing heat up to 450^{0}C until the drug is free from carbon and then cooled. This ash kept in a dessicator for 15-20 min. and weighed using Anamed Electronic balance, India and noted down the readings (Raghunathan, 1976) [9].

$$\textbf{\textit{Ash}}\ \% = \frac{\boldsymbol{B-C}}{\boldsymbol{A}} \times \mathbf{100}$$

Where, weight of empty crucible = C, weight of plant material = A, weight of crucible + ash = B, weight of ash = B-C.

Determination of Acid soluble ash
Total ash obtained was boiled for 15 min. in 25 ml of 25% hydrochloric acid and filtered to collect the insoluble matter on Whatman filter paper and ignited in a sintered crucible. It was allowed to cool and then kept in a desiccator for 15 min. The residue was weighed in a named Electronic balance and the acid soluble ash was calculated using the formula.

$$\textbf{\textit{Acid insoluble ash}}\ \% = \frac{\boldsymbol{B-C}}{\boldsymbol{A}} \times \mathbf{100}$$

Where, weight of empty crucible = C, weight of plant material = A, weight of crucible + ash = B, weight of ash = B-C.

Determination of Alcohol soluble extractive values

Macerate 4 gm of the air-dried drug, coarsely powdered, with 100ml of 90% ethanol in stoppered conical flask for 24 hrs, shaking the contents frequently during the first 6 hrs, thereafter filter rapidly taking precautions against loss of ethanol. Evaporate 25ml of the filtrate to dryness on water bath in a tarred flat bottom petri-plate. Dry at 105°C for 1 hr in hot air oven, cool in a desiccator and weigh repeat the process till the concurrent weight is obtained, calculate percentage of alcohol soluble extractive with reference to the air-dried drug.

$$\textit{Alcohol soluble Extractive}\ \% = \frac{B - C}{A} \times 4 \times 100$$

Where, weight of empty crucible = C, weight of plant material = A, weight of petri-plate + residue = B, weight of residue = B-C.

Determination of Water-soluble extractive values

Macerate 4g of air-dried drug, coarsely powdered, with 100 ml of chloroform water in a stoppered conical flask for 24 hrs, shaking the contents frequently during the first 6 hors. Thereafter filter rapidly by decanting the water extract. Evaporate the 25 ml of filtrate to dryness on a water bath in tarred flat bottom petri-plate or shallow dish. Add 2 ml of alcohol to the dry residue shake the contents and dry again on water bath. Dry at 1050 C for 1 hr. in hot air oven & cool in a desiccator for 30 minutes and weigh. Repeat the process till the concurrent weight is obtained; now calculate the percentage of water-soluble extractive of air-dried drug.

$$\textit{water soluble Extractive}\ \% = \frac{B - C}{A} \times 4 \times 100$$

Where, weight of empty crucible = C, weight of plant material = A, weight of petri-plate + residue = B, weight of extractive = B-C.

C. Powder microscopic studies

The powdered plant material was soaked in 20% Nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked plant material with saffranin and observed under microscope and the images were captured. (Johansen 1940) [10]

D. Thin layer chromatography

Place the sufficient quantity of homogenous mobile phase (different dilution factors) in to the chromatographic chamber to form a layer 5-6 mm deep. Close the chamber and allow to stand at constant room temperature, protected from direct sun light for 15 minutes.

Place the spots on the TLC plate, allow the spots to dry, place the plates as nearly vertical as possible in to the chamber, ensuring that the points of application are above the surface of the mobile phase. Close the chamber; develop the chromatogram at room temperature, allow the solvent to ascend the specified distance. Remove the plate, mark the position of the solvent front and allow the solvent to evaporate at room temperature. (E. Stahl-1965) [11]

3. Results and Discussion

A. Preliminary phytochemical tests

In the present study of preliminary phytochemical test on *Stevia rebaudiana* almost all the secondary metabolites were present which was shown by positive result. Whereas for saponins it was showing negative in foam test. (Table-1)

Phenols

Stevia rebaudiana responded positively to hot water test as indicated by the formation of brownish black ring at the junction of dipped and undipped portion of the leaf. ethanolic extract of *Stevia rebaudiana* responded positively to phenol test by producing intense colouration after adding with ferric chloride solution.

Flavonoids

The ethanolic extract of *Stevia rebaudiana* have shown positive response to flavonoid test by producing red coloration. Similarly, the positive response to Shinoda test by producing magenta colour indicating the presence of flavones in ethanolic extract of *S. rebaudiana.*

Steroids

Ethanolic extract of *S. rebaudiana* has shown positive response to Salkowski test by producing wine red ring at the junction of 2 layers but, Ethanolic extract of *S. rebaudiana* responded positively to Leiberman-Buchardt test by producing red colouration.

Triterpenes

Ethanolic extract of *S. rebaudiana* has indicated occurrence of triterpenes by responding positively to both Salkowski and Leiberman-Buchardt tests, by producing golden yellow colour after shaking vigorously and red ring respectively.

Diterpenes

Ethanolic extract of *S. rebaudiana* responded positively to copper acetate test by producing emerald green color.

Lactones

Ethanolic extract of *S. rebaudiana* responded positively to Fiegel's test and Baljet test by producing light pink and yellow colors respectively.

Tannins

Ethanolic extract of *S. rebaudiana* has responded positively to tannins by producing white precipitation.

Lignans

Ethanolic extract of *S. rebaudiana* responded positively to lignans by producing red color.

Saponins

Ethanolic extract of *S. rebaudiana* responded negatively.

Alkaloids

Ethanolic extract of *S.rebaudiana* responded positively by

producing creamy white, reddish brown, and orange red colored precipitation to Mayer's, Wagner's and Dragendorff's reagents respectively.

The results of preliminary phytochemical tests clearly indicated the presence of almost all groups/classes of the secondary metabolites in *S. rebaudiana*, except saponins.

Table 1: Indicating the occurrence of secondary metabolites in studied taxa

Sl No.	Tests	Observation
1.	Test for phenols a) Phenol test	+
2.	Test for flavonoids a) Shinoda test b) Flavonoids test	+ +
3.	Test for steroids a) Salkowski test b) Leiberman-Buchardt test	+ +
4.	Test for Triterpenes a) Salkowski test b) Leiberman-Buchardt test	+ +
5.	Test for Diterpenes a) Copper acetate test	+
6.	Test for lactones a) Feigels test b) Baljet test	+ +
7.	Test for tannins a) Tannin test b) Gelatin test	+ +
8.	Test for lignans a) Labat test b) Lignan test	+ +
9.	Test for saponins a) Foam test	-
10.	Test for alkaloids a) Mayer's test b) Wagner's test c) Dragendroff's test	+ + +

B. Physico chemical and Fluorescence Studies

One of the major problems encountered with the herbal product is quality of the drugs, toxic compounds, heavy metals, authentification of the specimen etc. There are several morphotypes and chemotypes of the plants are observed in natural condition since they exhibit phenotypic and chemotypic plasticity under such circumstances, the quality control of the raw drug plays an important role in standardization of herbal drugs. There are several such studies on pharmacognostic aspects of raw drugs.

Organoleptic characters

Organoleptic characters includes the character that one can feel with sensory organs. This character includes the color of the powdered drug, sensation, taste and oil or mucilaginous feeling. The details are indicated in the table-3.

Physical constant

The evaluation of drug basically needs its identification and can be done by morphological or microscopic characters. Even after identification it may be of substandard quality due to either incorrect collection or improper storage. Thus to prove its acceptability as drug it is important to know some physical constants such as Ash values and extractive values (Table-2).

Ash content

The ash which remains after incineration of drug may contain some inorganic compounds such as phosphates, carbonates, silicates and silica which are naturally occurring in drug or added deliberately in the form of adulterants. Many times crude drugs are mixed with mineral substances like sand, soil, calcium oxalate etc. An ash value is a criterion to judge the identity and purity. Hence, the study was under taken and the results are recorded.

Extractive values

The extracts obtained by exhausting crude drugs are identifying markers of their chemical constituents. Taking the chemical nature and properties of contents of drugs in consideration alcohol and water are used to determine the extractive values, as alcohol is an ideal solvent for extraction of various chemicals like tannins, alkaloids, resins etc. and in water many primary and secondary metabolites like tannins, sugars, plant acids, glycosides etc easily dissolves. The percent of extractive value of alcohol is (4.53%). Water soluble extractive value is 8.79%.

Table 2: Indicating the physical constants of *S. rebaudiana*

Sl. No.	Physical constant	S. rebaudiana (%)
1.	Total ash	9.35
2.	Acid soluble ash	1.53
3.	Alcohol soluble extractive	4.53
4.	Water soluble	8.79
5.	Loss on drying	0.9023g

Table 3: Showing organoleptic character of the taxa

Sl. No.	Character	*S. rebaudiana*
1.	Colour	Green
2.	Sensation	Smooth
3.	Taste	Sweet
4.	Oily stain	No
5.	Mucilaginous	No

C. Powder microscopic studies

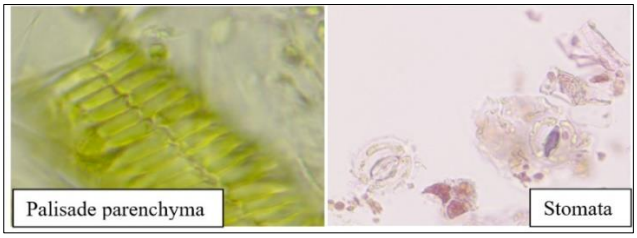

Fig 1

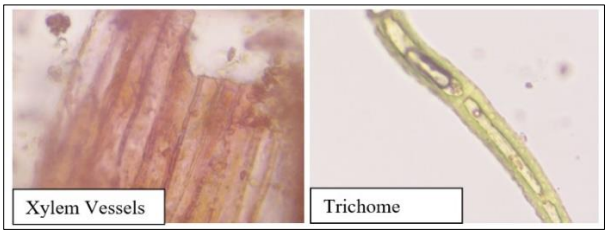

Fig 2

D. Thin layer chromatography
A=Ethyl acetate, B=Ethanol, C=Acetone, D=Water

1.Visible Light

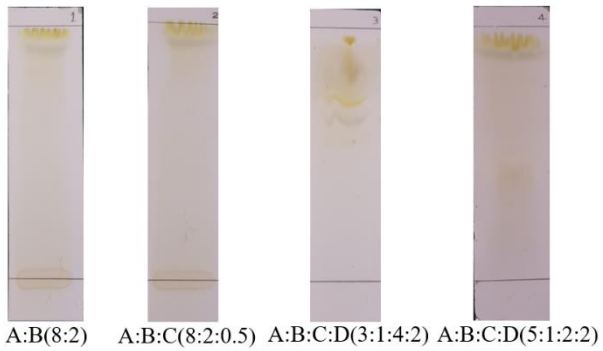

Fig 3

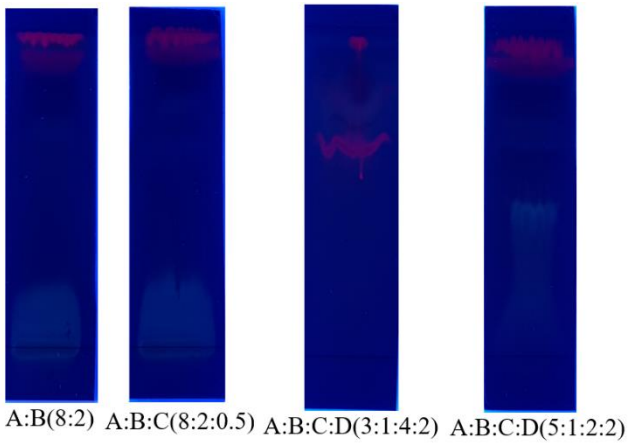

Fig 4

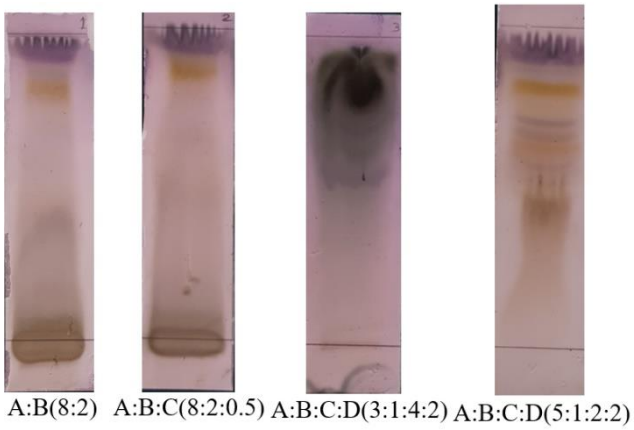

Fig 5

TLC can be used to help and determine the number of components in a mixture, the identity of compounds, and the purity of a compound in terms of bands. Different bands were found in each plates which are mentioned above and Rf values were calculated. In the visible light, found two bands which are having rf values 0.933 and 0.4. In the UV light, found three bands which are having Rf values 0.933, 0.4 and 0.66. And in vanillin sprayed plated, found six bands which are having Rf values 0.933, 0.8, 0.706, 0.66, 0.626 and 0.466.

4. Conclusion
The results indicated that S. rebaudiana has secondary metabolites such as phenols, flavonoids, alkaloids, steroids, tannins, triterpenes, diterpenes in them. But, saponins are absent in *S. rebaudiana*. The pharmacognostic studies revealed that the physical constants are reported in this plant for the first time as there are no other reports. The powder microscopic studies are helpful in authenticating the drug when raw drug leaf is supplied.

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