



Trade Science Inc.

ISSN : 0974 - 7508

Volume 6 Issue 4

# Natural Products

An Indian Journal

Full Paper

NPAIJ, 6(4), 2010 [196-200]

## Standardization of *Stevia rebaudiana* bertonii leaf and its herbal formulation (Bio-sweet tablet)

K.D.Desai\*, U.D.Shah, V.N.Shah

A.P.M.C.College of Pharmaceutical Education and Research, Himatnagar - 383 001, Gujarat, (INDIA)

Received: 15<sup>th</sup> November, 2010 ; Accepted: 25<sup>th</sup> November, 2010

### ABSTRACT

The present study was aimed at Standardization of the leaves of *Stevia rebaudiana* bertonii & its herbal formulation (Bio-sweet tablet). For standardization, physicochemical parameters were measured for the plant & formulation. The phytochemical-Stevioside have been isolated by developed isolation method from the leaves of *Stevia rebaudiana* & identified by spectral data. Also estimation of stevioside in plant & formulation was carried out using HPTLC. © 2010 Trade Science Inc. - INDIA

### INTRODUCTION

The process of evaluating the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observation is called standardization<sup>[1]</sup>. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation of its quality, safety and efficacy<sup>[2]</sup>. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. In India, the herbal drug market is about \$ one billion and the export of plant based crude drugs is around \$ 80 million. Also for the diabetes, herbal medicines are used worldwide so there is essential to standardize that formulation but the most important challenges faced by these formulations arise because of their lack of complete standardization. There may be batch to batch variation, quality control of raw material, pro-

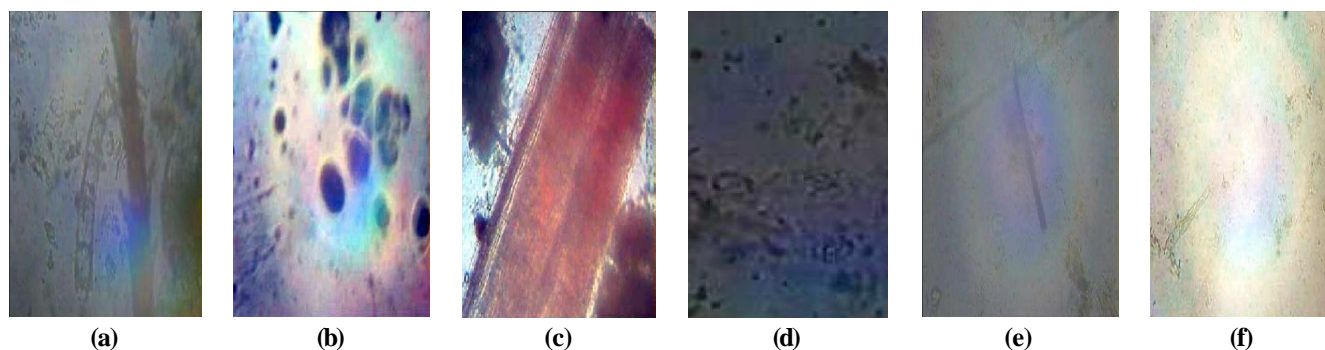
cess control and quality control of finished products. Several external factors like environmental, genetic, methods of cultivation, collection, harvest time, preparation, storage etc. also affects quality of herbal drugs. Hence standardization is essential for ensuring quality control of herbal drugs.

Each of 60mg Biosweet tablet contain, main ingredient Stevia leaf ext 15mg and remaining excipients (Additives) as per IP'96. It mainly used for Hypertension, Diabetes & Obesity.

*Stevia rebaudiana* bertonii (Asteraceae) is known as 'madhupatra' in Sanskrit in India, which is indigenous



Figure 1 : Photograph of leaves of *Stevia rebaudiana* bertonii



**Figure 2 : Powder study of *Stevia rebaudiana bertonii* leaves, (a) Simple trichomes with Bulbous terminal cells, (b) Starch grain, (c) Xylem vessel, (d) Anomocytic stomata, (e) Acicular fiber, (f) Simple trichomes with pointed apex**

to the northern regions of South America, between Brazil, Paraguay. It is grown commercially in many parts of Brazil, Paraguay, Uruguay, Central America, Israel, Thailand, India and China. Plant used as a natural sweetener, for diabetes, for high blood pressure, for cavity prevention as a weight loss aid<sup>[8]</sup>. Earlier reports on plant says it produces Anti-inflammatory & Immune Modulation Actions<sup>[10]</sup>, Hypoglycemic & Anti-diabetic Actions<sup>[11]</sup>, Hypotensive & Heart Tonic Actions<sup>[12]</sup> and Antimicrobial Actions<sup>[13]</sup>. Presence of flavonoids, tannin, alkaloids and sterols in *S. rebaudiana*<sup>[8]</sup>.

## MATERIAL AND METHODS

The formulation (Bio-sweet tablet) was collected from the sunrise remedies pvt LTD. Kalol. The crude material was collected from the LVG, Ahmedabad. The plant was authenticated & identified by Dr. Mukesh Prajapati H.N.S.B. LTD science college, Himatnagar. A voucher specimen has been preserved in the Department of Pharmacognosy, A.P.M.C.C.P.E.R., Himatnagar.

### Macroscopy

Leaves are perennial shrub that grows up to 1 m tall and has leaves 2-3 cm long green in color, characteristic in odor, sweet in test. They have alternative leaf arrangement and the shape of the surface of the leaves like Crenate (Figure 1).

### Microscopy

For microscopical studies, powdered leaves are clear with chloral hydrate solution. A drop of phloroglucinol and hydrochloric acid each was used to detect the lignified cells in powdered drug. It reveals common

characters of leaves such as Stomata, Trichomes, Xylem vessels and Fibers and starch grains<sup>[5,9]</sup> (Figure 2).

### Physicochemical parameters

Physicochemical parameters for raw material such as Moisture content / Loss on drying, Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive, Water soluble extractive & Determination of pH as per WHO guideline.

Physicochemical parameters for tablet such as Determination of pH, Disintegration test for Tablet, Friability test, Heavy Metal Analysis, Average weight, Weight variation test, Hardness test performed also as per Indian Pharmacopoeia<sup>[3,4]</sup>.

### Extraction of plant and preliminary phytochemical screening

The powdered leaf (100 g) of each plant kept in a thimble was extracted with 70% ethanol in a soxhlet extractor. Extraction process was continued until the color of the final drop of the extract became colorless. The extract was concentrated *in vacuo* at 60°C using a rotary evaporator. To evaporate the remaining solvent, the extract was kept in an oven at a temperature of 40-50°C for 8 hours. The extract was qualitatively evaluated for presence/absence of phytoconstituents such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones<sup>[6]</sup>.

### Isolation and identification of phytomarker

Isolation procedure for Stevioside was done by taking 1 kg of fresh leaves of *Stevia rebaudiana* extract it with 5 lit of hot water at 75°C for 4 hrs. Concentrate the above liquid up to 2 liters. Make pH 3 with

## Full Paper



**Figure 3 : Photograph of TLC isolated & standard marker**

50% citric acid, stirr constantly for 30 mins. Cool & filter. Filtrate heated at 50-55°C. Adjust pH 10.5 with calcium oxide. Agitated 60 min for 50-55°C. Cool and filter the precipitates. Clear yellow solution. Adjust pH 7.1 with 10% citric acid. Concentrate 250 ml. Syrupy mass triturate with n-butanol. Aqueous layer separated cool overnight for crystallization at 0-5°C. Dry in oven at 90°C. collect the yield of stevioside. Identify by single spot on TLC<sup>[7]</sup>, melting point & IR spectra. (Figure 3-5).

### High performance thin layer chromatography (HPTLC) studies

For estimation of stevioside from the plant and formulation done by taking the methanolic extracts of the both with known concentration. Former alcoholic solutions were taken and quantified by HPTLC using n-Butanol : Glacial acetic acid : water (8 : 2 : 2)<sup>[7]</sup> as mobile phase and Silica Gel 60F<sub>254</sub>, as stationary phase. The plates were scanned at 450 nm after derivatization. Concentration of stevioside was calculated using their respective calibration curve generated from the peak area of concentration plot. Derivatization was done with methanolic sulphuric acid reagent followed by heating at 110°C for 10 min. HPTLC was done by employing Camag linomat V-semiautomatic spotting device, Hamiton 100µl HPTLC syringe, Camag twin-trough chamber, Camag TLC Scanner 3 and Camag WINCATS integration software (Figure 6).

## RESULT AND DISCUSSION

Powder of Stevia dry extract was dark brown, odor was characteristic & taste was sweet. It was easily iden-

tified by their odor and taste.

Powder microscopy study indicating dense masses of starch grain and few very small individual granules scattered as such; it contain main character with their microscopy of leave epidermis are different type of trichomes among them there are simple trichomes with bulbous cells and simple trichomes with pointed apex were identified; they have highly lignified pitted xylem vessels; also the second epidermal character was stomata contain anomocytic type; also in lignified section they have acicular fiber with pink stain (Figure 2).

Ingredient of Bio-sweet tablet, Stevia raw material has moisture content 2.4. Moisture content of ingredient Bio-sweet tablet was within the limit (<5% w/w). pH of extract was near about neutral 5.8 (Slightly acidic). Results suggest that moisture content & pH of extract of plant was comparable and within the limit.

Alcohol soluble extractive was found 30.5% in raw material and Water soluble extractive value was 36.5% in raw material. Water soluble extractives were higher than Alcohol soluble extractives in raw material of Bio-sweet tablet.

Total ash values (11% w/w), Acid insoluble ash (3.5% w/w) & Water soluble ash (1.13% w/w) were determined as per WHO. Acid insoluble ash value was higher compare with water soluble ash. It indicates that acid insoluble inorganic materials are more present then the other in Bio-sweet tablet.

For phytochemical screening, result indicated the presence of flavonoids, tannin, alkaloids, carbohydrates and sterols in *S. rebaudiana*. & formulation (TABLE 1).

Bio-sweet tablet contains Color (White), Taste (sweet), Disintegration time (6.5 min), Friability (pass), Hardness (1.0kg/cm<sup>2</sup>), pH (6.7), weight variation (Pass). All this value within the limit given in IP'96.

In Heavy metal analysis concentration of lead in Bio-sweet tablet was 1.87 ppm respectively. Cadmium, Mercury and lead were below limit of detection the formulation. This indicates that Bio-sweet tablet passes the limit for heavy metal.

Compound was isolated from water extract of *Stevia rebaudiana bertonii* leaves and characterized by Melting point, TLC & FT-IR. Melting point (197-199°C), (880 cm<sup>-1</sup>, 1395 cm<sup>-1</sup>, 1725 cm<sup>-1</sup>, 2950 cm<sup>-1</sup>, 3400 cm<sup>-1</sup>) (Figure 4 & 5). FT-IR peaks & Single spot

**TABLE 1 : Qualitative phytochemical evaluation of Bio-sweet tablet & its ingredient**

Test	Reagents	<i>S.rebaudiana</i> formulation	
Test for alkaloids	Dragendorff's	++	++
	Mayer's	++	++
Test for steroidal compounds	Acetic anhydride and conc. sulfuric acid	+++	+++
	Chloroform and conc. sulfuric acid	+++	+++
Test for Phenolic compounds	Ferric chloride and potassium ferrocyanide	++	++
	10% Lead acetate	++	++
Test for Flavonoids	Sodium hydroxide	++	++
	Ethyl acetate	++	++
Test for Saponnins	Froth test	±	±
Test for Tannins	Ferric chloride	±	±
	Lead acetate	±	±
Test for Glycoside	Cardiac glycosides	-	-
	Test for free anthraquinones	-	-

**Key: +++: Very strong positive, ++: Strong positive, ± : Trace, - : Negative**

**TABLE 2 : IR Peak table for standard & Isolated compound**

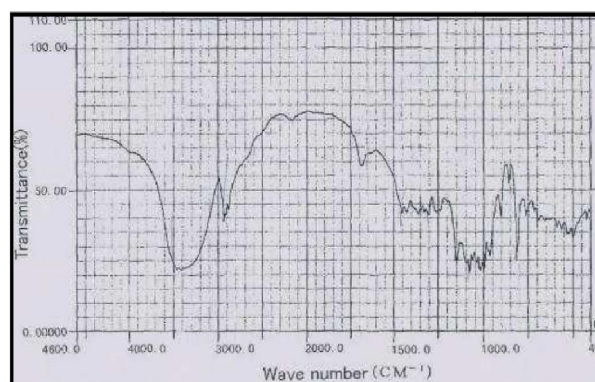
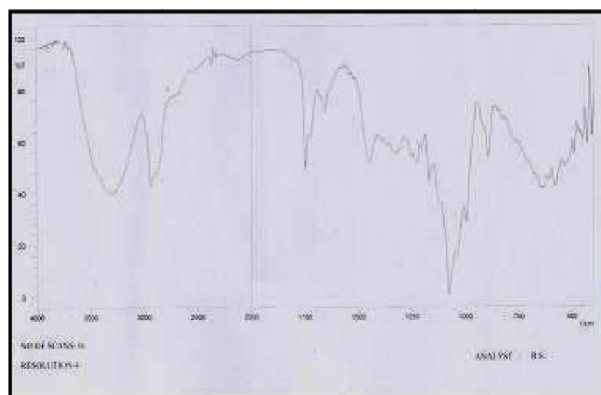
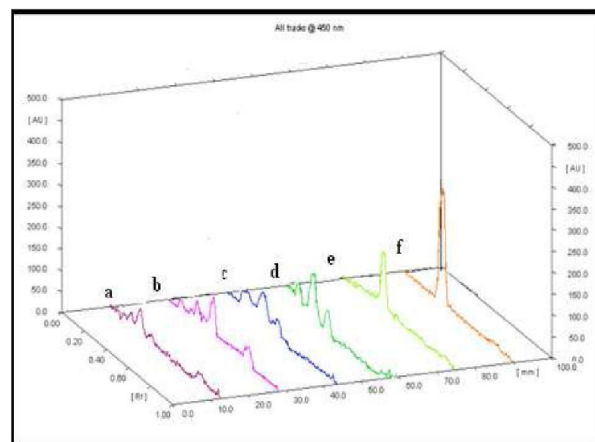
IR Spectrum peaks (cm <sup>-1</sup> )		Peak Identification
Isolated	Standard	
880 cm <sup>-1</sup>	885 cm <sup>-1</sup>	=C-H & =CH <sub>2</sub> stretching vibration of alkenes groupe
1395 cm <sup>-1</sup>	1400 cm <sup>-1</sup>	-C-O-H bending vibration of acidic groupe
1725 cm <sup>-1</sup>	1720 cm <sup>-1</sup>	-C=O bending vibration of saturated ketone groupe
2950 cm <sup>-1</sup>	2950 cm <sup>-1</sup>	-CH <sub>3</sub> stretching vibration of alkane groupe
3400 cm <sup>-1</sup>	3400 cm <sup>-1</sup>	-OH stretching vibration of alcohols & phenol groupe

**TABLE 3 : HPTLC for stevioside in plants and formulation**

Name of sample	Plant	formulation
Concentration (ng)	6000 ng	6000 ng
Peak area	4674	3624
Avg amt of stevioside±SD (m=3)	3.4199 ± 4.5	1.8372 ± 3.1
Avg % w/w ± SD	0.05899 ± 0.0005	0.030 ± 0.0003
%CV	0.09	0.085

on TLC plate at 0.3 R<sub>f</sub> perfectly match with standard stevioside indicates may be presence of stevioside (Figure 2 & TABLE 2).

Stationary phase Silica gel TLC plate and mobile phase n-Butanol; Glacial acetic acid; water (8:2:2) had given good separation of Stevioside at R<sub>f</sub> = 0.30. The detector response/calibration curve of Stevioside was found to be linear dependent on the concentration against area. The best fitting line equation was  $y = 663.4x +$

**Figure 4 : IR spectrum of standard compound****Figure 5 : IR spectrum of isolated compound****Figure 6 : HPTLC analysis of plant *Stevia rebaudiana bertonii* (a & b), formulation (c & d) and standard stevioside (e & f). Three D image after derivatization (450nm)**

2405.2 (R<sup>2</sup> = 0.9992). Correlation coefficient 0.9992 indicated good linearity between concentration and peak area. Stevioside content in the methanolic extract of leaves of *Stevia rebaudiana bertonii* and Formulation by the proposed HPTLC method was found to be 0.056 % w/w and 0.030% w/w respectively (Figure 6 & TABLE 3). The identity of the Stevioside band at 0.3

## Full Paper

$R_f$  in the sample extract solution was confirmed by overlaying/superimposing the UV absorption spectrum of the sample with that from the reference standard of Stevioside, using the camag TLC scanner 3. The lowest detectable limit of Stevioside was found up to 3 ng, and provides good resolution and separation of Stevioside from other constituents of *Stevia rebaudiana bertonii*. Further, recovery values of Stevioside were found to be about 99%, which shows the reliability and suitability of the method.

We concluded that plant *Stevia* is sweet in test and use in diabetes due to their anti-diabetic activity. In this research we have isolated the phytomarker compound—Stevioside and established TLC densitometric method for the quantification of Stevioside from *Stevia rebaudiana bertonii* plant and Bio sweet tablet—a herbal formulation using HPTLC. In our study we found, Stevioside content in plant (0.056% w/w) and formulation (0.030% w/w). The method was found to be simple, precise, specific, sensitive and accurate and can be used for its quantification in the plant materials and also in routine quality control of the raw materials as well as formulations containing this compound.

### ACKNOWLEDGEMENTS

The author are thankful to the Mr Manshukhbhai Dhadani (Managing Director, Sunrise Remedies pvt LTD, Gandhinagar.) for providing facilities for research work.

### REFERENCES

- [1] R.N.Chopra, S.L.Nayar, I.C.Chopra; 'In Glossary of Indian Medicinal plants', Council of Scientific and Industrial Research, New Delhi, **1**, 197 (1956).
- [2] P.Mohapatra, A.Shirwaikara, H.N.Asawtharam; Pharmacognosy Magazine, **4(13)**, 65-69 (2008).
- [3] World Health Organization, 'Quality Controls Methods for Medicinal Plant Materials', Geneva, AITBS Publisher and Distributors, Delhi, 8-70 (2002).
- [4] 'Indian Pharmacopoeia', The Controller of Publication, Delhi, **2**, A-95,736, A-81-83 (1996).
- [5] V.Rajpal; 'Standardization Of Botanicals', Testing & Extraction Methods of Medicinal Herbs, Eastern Publishers, India, **1**, 140-148 (2002).
- [6] K.R.Khandelwal; 'Practical Pharmacognosy', 2<sup>nd</sup> Ed., Nirali Prakasan, 149 (2001).
- [7] S.Kumar, N.J.Lincroft; Journal of Natural Product, **2**, 6-7 (1986).
- [8] V.J.Brondegaard; Planta Med., **23**,167-172 (1973).
- [9] M.Chauhan, A.P.G.Pillai; 'Microscopic Profile of Powdered Drugs used in Indian Systems of Medicine', Nirali Prakashan, Pune, **2**, 134 (2007).
- [10] C.Boonkaewwan; J.Agric.Food Chem., **54(3)**, 785-9 (2006).
- [11] T.H.Chen; Planta.Med., **71(2)**, 108-13 (2005).
- [12] L.A.Ferri; Phytother.Res., **20(9)**, 732-6 (2006).
- [13] T.Matsukubo; Int.Dent.J., **56(3)**, 119-30 (2006).