Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 17/06/2025
Revised: 27/06/2025
Accepted: 29/06/2025
Published: 08/07/2025

Jay Prakash Singh, Shikha Sharma
Lords University, Alwar Rajsthan-301001.

Correspondence

Jay Prakash Singh
Lords University, Alwar Rajsthan-301001.

Email: jpsingh9452@gmail.com

DOI: 10.24092/CRPS.2025.150204

Website: www.crpsonline.com

Quick Response Code:

PHYTOCHEMICAL SCREENING, PHARMACOGNOSTICS CHARACTERIZATION, AND CHROMATOGRAPHIC ANALYSIS (TLC/HPTLC) OF *STEVIA REBAUDIANA* BERTONI LEAF EXTRACT

Jay Prakash Singh, Shikha Sharma

ABSTRACT

The growing demand for natural sweeteners as alternatives to synthetic sugars has heightened interest in *Stevia rebaudiana* Bertoni, a perennial shrub native to South America, known for its high-intensity, non-caloric steviol glycosides. This study aimed to evaluate the pharmacognostic, phytochemical, and chromatographic properties of *S. rebaudiana* leaves to establish quality control parameters and validate its therapeutic potential. Fresh leaves were authenticated, shade-dried, and subjected to ethanolic extraction via cold maceration. Macroscopic and microscopic analyses revealed characteristic features such as lanceolate leaves with serrated margins, anisocytic stomata, and calcium oxalate crystals. Physicochemical evaluation showed acceptable moisture content (6.2%), total ash (9.4%), and extractive values (14.3% water-soluble). Phytochemical screening confirmed the presence of steviol glycosides, flavonoids, phenolics, and terpenoids, supporting its antioxidant and antidiabetic properties. Chromatographic profiling using TLC and HPTLC resolved key markers, with stevioside identified at Rf 0.67 (TLC) and 0.65 (HPTLC), providing a reliable fingerprint for standardization. The findings underscore *S. rebaudiana*'s potential as a natural sweetener and therapeutic agent, while establishing quality benchmarks for its use in nutraceutical and pharmaceutical applications. Further clinical studies are recommended to explore its efficacy and safety.

Key words: *Stevia rebaudiana*, pharmacognostic evaluation, phytochemical screening, HPTLC fingerprinting, steviol glycosides, natural sweetener.

1 INTRODUCTION

The increasing demand for natural sweeteners as alternatives to synthetic sugars has driven scientific interest in *Stevia rebaudiana* Bertoni, a perennial shrub native to South America.[1] This plant is renowned for its steviol glycosides, particularly stevioside and rebaudioside A, which exhibit high sweetness intensity (200–300 times sweeter than sucrose) while being non-caloric and non-cariogenic Beyond its sweetening properties, *S. rebaudiana* possesses antioxidant, antidiabetic, antimicrobial, and anti-inflammatory activities, making it a valuable candidate for nutraceutical and pharmaceutical applications[1].

Despite its widespread use, the standardization of *S. rebaudiana*-based products remains a challenge due to variations in phytochemical composition influenced by cultivation conditions, extraction methods, and plant maturity.[1] Phytochemical screening and pharmacognostic characterization are essential for ensuring botanical authenticity, purity, and quality control of herbal materials (WHO, 2011). Additionally, chromatographic techniques such as thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) provide rapid, reliable, and cost-effective methods for fingerprinting and quantifying bioactive compounds.[2]

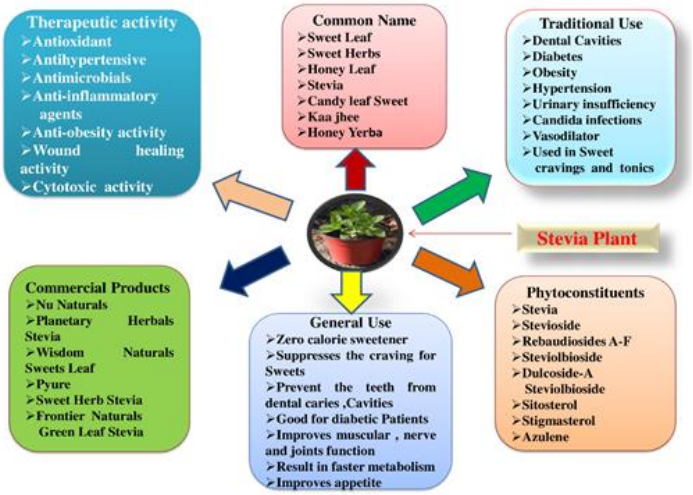

Figure 1: Traditional application of stevia leaf[3]

2 MATERIALS AND METHODS

2.1 Plant Material Collection And Authentication

Bertoni *Stevia Rebaudiana* Plant Purchased from CIMAP Lucknow and Cultivation and Harvesting in Our home Garden After the one month Grown the plant leaf then sample Leaf will be Collected very carefully and they are taken some days for normal dry and the sample will be sent to NBRI Lucknow After Authentication We are collect the plant Authentication Certificate. Plant Authenticated by Dr. K.M. Prabhu Kumar senior Scientist CSIR-NBRI Lucknow Uttar Pradesh. Plant Authentication Certificate No-PDSH/LWG/Authentication/2023-24/17.

2.2 Preparation of Plant Extract

The collected leaves were washed thoroughly with distilled water to remove dirt and debris, shade-dried at room temperature for 7–10 days, and then powdered using a mechanical grinder. The powdered material was stored in an airtight container for further use.

Extraction was performed using the cold maceration method. About 50 g of the powdered leaf material was soaked in 250 mL of ethanol on solvent for 72 hours with occasional stirring. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The dried extract was stored in a desiccator until use [4].

3 PHARMACOGNOSTICS CHARACTERIZATION

3.1 Macroscopic Evaluation

The fresh leaves of *stevia rebaudiana* were examined for or-Ganoleptic characteristics such as colour, size, shape, odour, taste, and texture.

Table 1: Macroscopic Evaluation of *Stevia rebaudiana* [5]

Parameter	Description
Shape	Lanceolate to elliptic or ovate-lanceolate
Size	2–7 cm long, 0.5–1.5 cm wide (mature leaves)
Apex	Acute to slightly acuminate
Base	Attenuate or cuneate
Margin	Serrate (coarsely toothed, 2–6 teeth per cm)
Venation	Pinnate, with prominent midrib and secondary veins
Surface	Upper: Glabrous (smooth), dark green; Lower: Slightly pubescent, lighter green
Texture	Thin but firm, slightly brittle when dry
Petiole	Short (3–10 mm), grooved, light green to reddish
Arrangement	Opposite decussate
Aroma	Slight herbal scent (intensifies when crushed)
Taste	Intensely sweet (due to steviol glycosides)
Glands	Presence of trichomes (especially on veins and margins)

3.2 Microscopic Evaluation

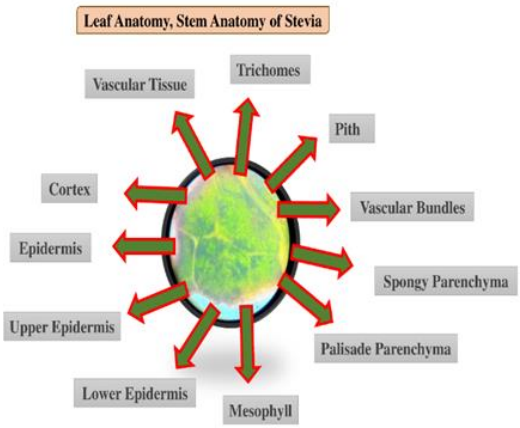

Figure 2: Leaf and Stem Anatomy of *Stevia*[7]

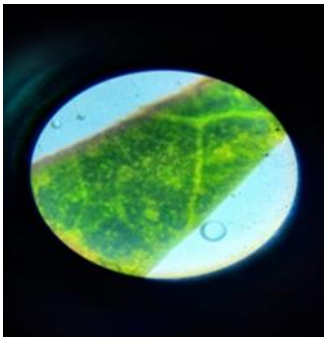

Figure 3: T.S of *Stevia* leaf

3.3 Physicochemical Parameters

Standard procedures were followed to determine the following parameters:

- Moisture content (loss on drying)
- Ash values (total ash, acid-insoluble ash, water-soluble ash)
- Extractive values (alcohol-soluble and water-soluble extractives)
- Foreign organic matter

These tests were performed according to standard guidelines mentioned in WHO or Indian Pharmacopoeia.[8]

4 PRELIMINARY PHYTOCHEMICAL SCREENING

The methanolic extract of *Stevia rebaudiana* leaves was subjected to qualitative phytochemical tests to detect the presence of major classes of phytoconstituents using standard methods:

- Alkaloids – Mayer's, Dragendorff's test
- Flavonoids – Alkaline reagent test
- Tannins and Phenolics – Ferric chloride test
- Saponins – Foam test
- Terpenoids – Salkowski's test
- Glycosides – Keller-Kiliani test
- Steroids – Liebermann-Burchard test

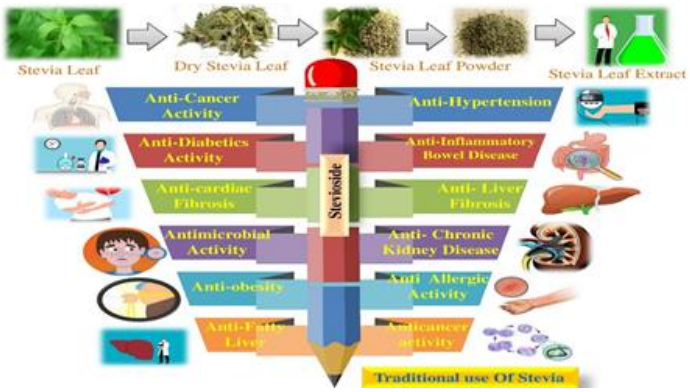

Figure 4: Extraction and Identification of Stevioside with Crucial Therapeutic Action[9]

5 CHROMATOGRAPHIC ANALYSIS

5.1 Thin Layer Chromatography (TLC)

TLC was performed on pre-coated silica gel plates. The methanolic extract was spotted using a capillary tube and developed in a suitable mobile phase Ethyl acetate: Methanol: Water (80:10:10) The developed plates were visualized under UV light (254 nm and 366 nm) and after spraying with specific detecting reagents (anisaldehyde-sulfuric acid)[10].

Figure 5: Extraction by Soxhlet Apparatus

Figure 6: Identification Test

Figure 7: TLC plate[11]

5.2 High-Performance Thin Layer Chromatography (HPTLC)

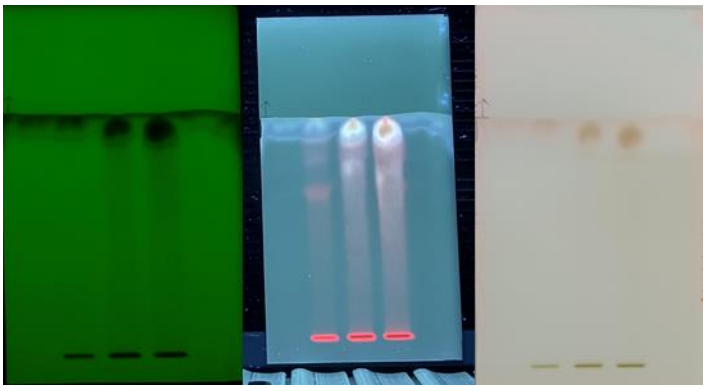

Figure 8: Plate under 254 nm

Figure 9: Plate under 366 nm

Figure 10: plate under white light

HPTLC analysis was carried out using an automatic sample

applicator and a HPTLC system. The extract was applied as bands, and the chromatogram was developed in a selected mobile phase. Densitometric scanning was performed at specific wavelengths (254 nm or 366 nm) to obtain chromatographic fingerprints. The retention factor (Rf) values 0.50–0.67 and peak profiles were recorded and analysed.[12]

5.2.1 HPTLC Finger Print Profile of ethanolic extract of Stevia rebaudiana

Application: Linomat 5 Application (camag)
Volume applied: 10µl
Solvent System: Ethyl acetate: methanol: water (7.5:1.5:1.0)
Scan Wavelength: 254 nm, 366 nm
TLC Plate Development: Pre-saturated Camag Twin Through Chamber[13]

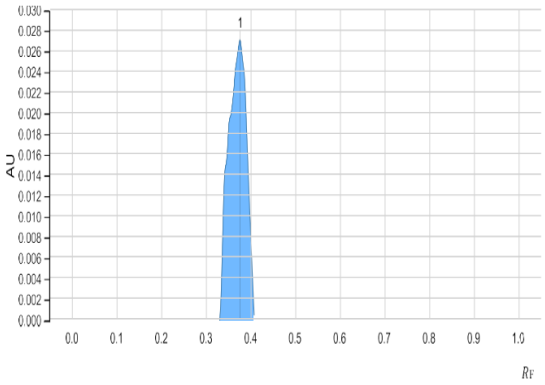

Figure 11: HPTLC Chromatogram of (Ethanol Extract) 01

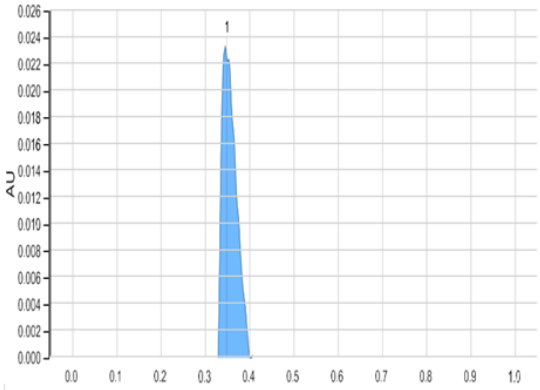

Figure 12: HPTLC Chromatogram of (Ethanol extract)02

6 REAGENTS AND INSTRUMENTS

- Solvents: Methanol, ethanol, chloroform, hexane, distilled water (analytical grade)
- Instruments: Compound microscope, TLC chamber, rotary evaporator, UV chamber, HPTLC system, electronic balance.

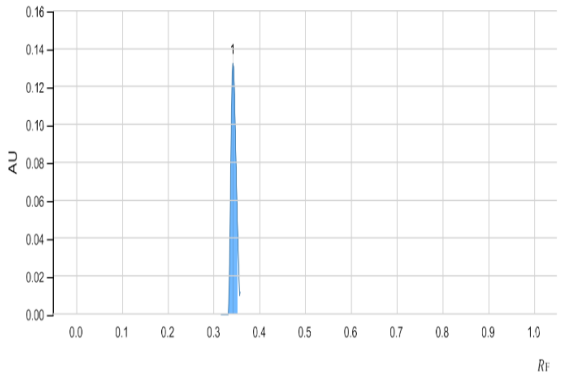

Figure 13: HPTLC Chromatogram of (Ethanol Extract)03

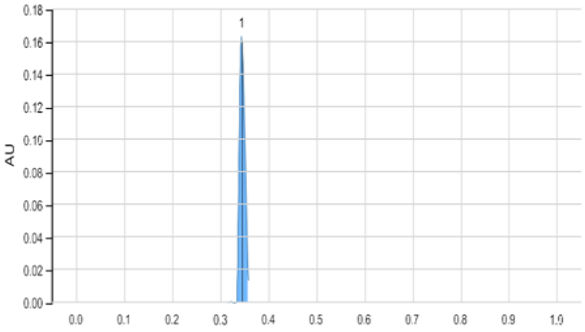

Figure 14: HPTLC Chromatogram of (Ethanol Extract)04 [15]

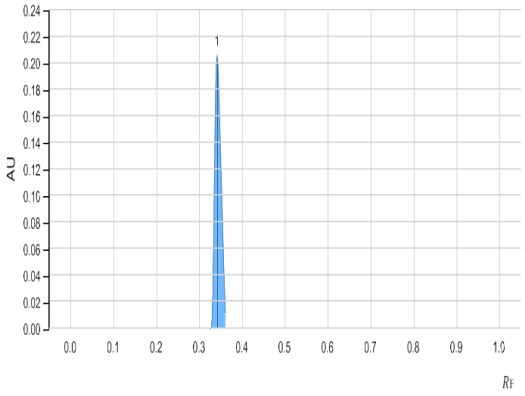

Figure 15: HPTLC Chromatogram of (Ethanol Extract)05

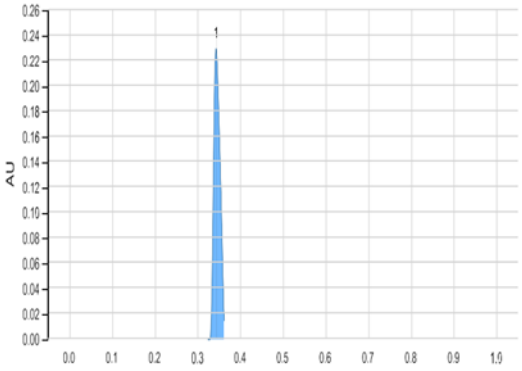

Figure 16: HPTLC Chromatogram of (Ethanol Extract) 06 [16]

7 RESULTS AND DISCUSSION

7.1 Pharmacogenetics Characterization

7.1.1 Macroscopic analysis

The fresh leaves of *Stevia rebaudiana* were observed to be green, ovate to lanceolate in shape, and slightly serrated along the margins. The leaves had a characteristic mild odour and a sweet taste due to the presence of steviol glycosides [17].

7.1.2 Microscopic analysis

Microscopic evaluation of the transverse section of *Stevia rebaudiana* leaf revealed key anatomical features such as:

- Bicollateral vascular bundles
- Uniseriate epidermis with multicellular trichomes
- Parenchymatous mesophyll with calcium oxalate crystals
- Anisocytic stomata, mainly on the abaxial surface

Powder microscopy showed fragments of epidermal cells, trichomes, xylem vessels, and crystals of calcium oxalate, which are characteristic diagnostic features of *Stevia rebaudiana*.

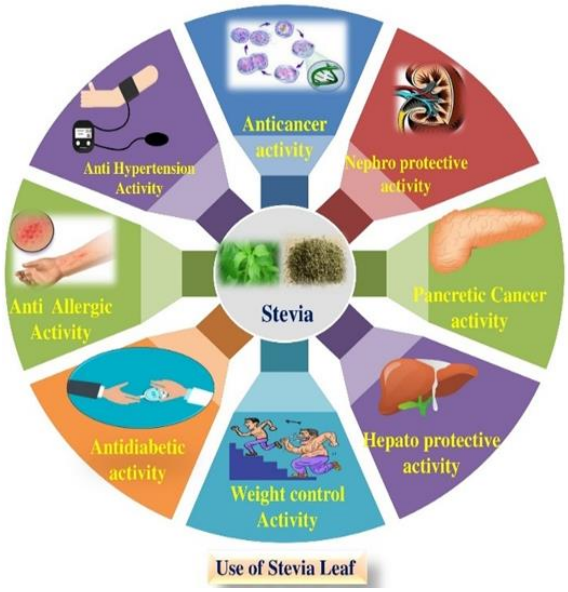

Figure 17: Application of Stevia leaf [18]

7.1.3 Physiochemical parameters

The physicochemical evaluation of the powdered leaf material revealed the following values:

Table 2: These values are within the acceptable limits for herbal raw materials and serve as quality control parameters for future standardization.[19]

Parameter	Observed Value (%)
Loss on drying	6.2
Total ash	9.4
Acid-insoluble ash	1.2
Water-soluble ash	4.7
Alcohol-soluble extractive	8.1
Water-soluble extractive	14.3
Foreign organic matter	Absent

7.2 Phytochemical Screening

Preliminary phytochemical analysis of the methanolic leaf extract of *Stevia rebaudiana* revealed the presence of several bioactive constituents. The strong presence of flavonoids, phenolic compounds, and glycosides, particularly steviol glycosides, supports the sweetening and antioxidant properties of *Stevia rebaudiana*. These compounds also contribute to its pharmacological actions, such as anti-inflammatory, anti-hyperglycaemic, and antimicrobial effects.[20]

Table 3: Preliminary phytochemical analysis of the methanolic leaf extract of *Stevia rebaudiana*

Phytochemical Constituents	**Test Result**
Alkaloids	+
Flavonoids	+++
Tannins	++
Saponins	+
Terpenoids	++
Steroids	+
Glycosides (Steviol)	+++
Phenolic compounds	+++

(+: Present, ++: Moderately Present, +++: Abundant)

7.3 Chromatographic Analysis

7.3.1 Thin layer chromatography (TLC)

The TLC analysis revealed multiple distinct bands under UV light, indicating the presence of various phytochemical components. The best separation was achieved using the solvent system Ethyl acetate: Methanol: Water (80:10:10).[21]

Several spots were observed with different Rf values. Notably:

- A major band corresponding to Stevioside was visible with a Rf of approximately 0.67.

- Additional bands indicating the presence of flavonoids and phenolics were also seen at lower Rf values.

7.3.2 High-Performance Thin Layer Chromatography (HPTLC)

HPTLC fingerprinting of the methanolic extract provided a more detailed profile. The densitogram at 254 nm and 366 nm revealed well-resolved peaks, suggesting the presence of multiple phytoconstituents. Major peaks were observed at Rf values of 0.24, 0.45, 0.65, and 0.82, with the most prominent peak at 0.65, corresponding to steviol glycosides.

These profiles can serve as reliable fingerprints for the identification and quality control of *Stevia rebaudiana* extracts in herbal formulations.[22]

8 SUMMARY

This study comprehensively evaluated the pharmacognostic, phytochemical, and chromatographic properties of *Stevia rebaudiana* Bertoni leaf extract to establish its identity, purity, and potential therapeutic applications. The research employed a multi-faceted approach, integrating macroscopic, microscopic, physicochemical, and advanced chromatographic techniques to validate the plant's traditional use and support its standardization for nutraceutical and pharmaceutical purposes.

8.1 Pharmacognostic Characterization

- Macroscopic features: Leaves exhibited lanceolate shape, serrated margins, pinnate venation, and a sweet taste due to steviol glycosides.
- Microscopic analysis: Identified diagnostic markers like anisocytic stomata, multicellular trichomes, and calcium oxalate crystals**.**

8.1.1 Physicochemical Parameters

- Moisture content (6.2%), total ash (9.4%), and extractive values (e.g., 14.3% water-soluble) aligned with pharmacopoeial standards, ensuring material quality.

8.2 Phytochemical Screening

- The methanolic extract revealed abundant steviol glycosides (stevioside, rebaudioside A), flavonoids, phenolic compounds, tannins, and terpenoids.
- These bioactive constituents underpin the plant's antioxidant, anti-diabetic, and anti-inflammatory properties.

8.3 Chromatographic Profiling (TLC/HPTLC)

- TLC: Solvent system (ethyl acetate: methanol: water, 80:10:10) resolved stevioside at Rf ~0.67, with additional bands for flavonoids and phenolics.
- HPTLC: Peaks at Rf 0.24, 0.45, 0.65 (major stevioside peak), and 0.82 provided a reproducible fingerprint for quality control.

9 CONCLUSIONS

- The study confirms the botanical authenticity and phytochemical richness of *Stevia rebaudiana*, validating its use as a natural sweetener and therapeutic agent.
- Standardization tools: Microscopic features and chromatographic fingerprints (e.g., stevioside at Rf 0.65) serve as reliable markers for authentication and regulatory compliance.
- Future directions: *In vivo* studies and clinical trials are recommended to explore dose efficacy, safety, and broader pharmacological applications.

REFERENCES

1. An HJ, & Jin H. Physicochemical standardization of herbal materials: Methods and applications. Journal of Pharmacognosy and Phytochemistry. 2012;1(3):1-12.
2. Ceunen S, & Geuns JM. Steviol glycosides: Chemical diversity, metabolism, and function. Journal of Natural Products. 2013;76(6):1201-1228. https://doi.org/10.10 21/np400203b
3. Chatsudthipong V, & Muanprasat C. Therapeutics, Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacology & Therapeutics. 2009;121(1):41-54. https://doi.org/10.10 16/j.pharmthera.2008.09.007
4. Geuns JM. Sativoside. Phytochemistry. 2003;64(5):913-921. https://doi.org/10.1016/S0031-9422(03)00426-6
5. Goyal SK, Samsher S, & Goyal RK. Stevia *(Stevia rebaudiana)* a bio-sweetener: A review. International Journal of Food Sciences and Nutrition. 2010;61(1):1-10. https://doi.org/10.3109/09637480903193049
6. Kaushik R, Narayanan P, Vasudevan V, Muthukumaran G, & Antony U. Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of

leaf extracts. Journal of Food Science and Technology. 2010;47(1):27-33. https://doi. org/10.1007/s13197-010-0011-7

7. Lemus-Mondaca R, Vega-Gálvez A, Zura-Bravo L, & Ah-Hen K. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional, and functional aspects. Food Chemistry. 2012;132(3):1121-1132. https://doi.org/10.1016/j.foodchem.2011.11.140
8. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. Lancet. 2021;397(10281):1301-1315.
9. Singh SD, & Rao GP. Stevia: The herbal sugar of the 21st century. Sugar Tech. 2005;7(1):17-24. https://doi.org/10.1007/BF02942413
10. Tiwari S. Pharmacognostic and phytochemical evaluation of Stevia rebaudiana Bertoni leaves. Asian Journal of Pharmaceutical and Clinical Research. 2010;3(3):224-226.
11. Wagner H, & Bladt S. Plant drug analysis: A thin layer chromatography atlas (2nd ed.). Springer.1996.
12. Yadav AK, Singh S, Dhyani D, & Ahuja PS. A review on the improvement of stevia [*Stevia rebaudiana* (Bertoni)]. Canadian Journal of Plant Science. 2011;91(1):1-27. https://doi.org/10.4141/cjps10086
13. Chaturvedi P, & George S. HPTLC analysis of steviol glycosides in *Stevia rebaudiana*. Journal of Planar Chromatography. 2013;26(3):226-230.
14. JECFA (Joint FAO/WHO Expert Committee on Food Additives). Safety evaluation of steviol glycosides. WHO Food Additives Series, 2010;60: 183-218.
15. Kim IS, Yang M, Lee OH, & Kang SN. The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT-Food Science and Technology. 2011;44(5):1328-1332.
16. Mizutani K, & Tanaka O. Use of *Stevia rebaudiana* sweeteners in Japan. In Stevia: The genus Stevia. CRC Press. 2002;178-195.
17. Prakash I, DuBois GE, Clos JF, Wilkens KL, & Fosdick LE. Development of rebiana, a natural, non-caloric sweetener. Food and Chemical Toxicology. 2008;46(7):S75-S82.
18. Shivanna N, Naika M, Khanum F, & Kaul VK. Antioxidant, anti-diabetic, and renal protective properties of *Stevia rebaudiana*. Journal of Diabetes and Its Complications. 2013;27(2):103-113.
19. Tadhani MB, Patel VH, & Subhash R. *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. Journal of Food Composition and Analysis. 2007;20(3-4):323-329.
20. Wolwer-Rieck U. The leaves of *Stevia rebaudiana (*Bertoni), their constituents, and the analyses thereof: A review. Journal of Agricultural and Food Chemistry. 2012;60(4):886-895.
21. Yoda SK, Ferreira ME, & Pessoa OD, Supercritical fluid extraction from Stevia rebaudiana Bertoni using CO_2 and CO_2+water: Extraction kinetics and identification of extracted components. Journal of Food Engineering. 2003;57(2):125-134.
22. Zhang Q, Yang H, Li Y, Liu H, & Jia X. Toxicological evaluation of ethanolic extract from *Stevia rebaudiana Bertoni* leaves: Genotoxicity and subchronic oral toxicity. Regulatory Toxicology and Pharmacology. 2017;86:253-259.