



Isolation and Extraction of Artificial Sweetener (Stevia) Using Stevioside Conventionally

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KEYWORDS

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ABSTRACT:

Introduction: The herb The Bertoni Stevia rebaudiana family (asteraceae or compositae) is used to extract stevioside from its leaves. It serves as a natural, minimal-calorie sweetener because of its pleasant flavour. These glycosides have a broad range of medicinal applications and possess 300 times the sweetness of sugar. Furthermore, Dietary carbohydrates are abundant in stevia plants. (60.92% d.w.), amino acid (12.42% d.w.), raw cellulose (14.53% d.w.), elements (a K-22.16; Ca-16.6; The sodium-15.92; Manganese-3.25 mg/100gm d. wright.; Copper-0.73; Manganese-2.89; the iron-5.89; Zn-1.25 mg/100gm d.w.), plus cholesterol. In many places, it is used in place of sucrose in food and beverages.

Objectives: Identify natural phytoconstituents that can be use for sweetening agent in Bertoni Stevia rebaudiana.

Methods: Isolation of Bertoni Stevia rebaudiana done by Soxhlet apparatus and then the extract undergoes TLC Chromatography study for isolation

Results: Using two distinct solvents with Rf values of 0.50 and 0.67 on TLC, stevioside was effectively separated. It was then further purified and recrystallized.

Conclusions: - The low-calorie steviol glycosides called steviosides are found in its Rebaudiana Bertoni the plant's leaflets. They are frequently used by diabetic people as a natural sweetener. The stevioside band is visible in the TLC data, and this is verified by contrasting the samples' Rf value (0.50) with the standard's.

1. Introduction

Natural non-nutritive sweeteners that have a broad variety of medicinal applications [1], as well as essential amino acids [2] with minimal calorific worth as a substitute for dextrose [1], have become increasingly in demand in recent years. The most prevalent and rapidly growing health issue in India and many other nations is diabetes. By 2025, there will be 70 million diabetics worldwide, up from 40 million at present, predicts IDF. By 2030, India will have the greatest number of diabetics, followed by China and the United States[3]. Phytochemistry is the study of the chemistry of plants. The perennial plant-based product, the species Stevia rebaudiana Bertoni, is an Asteraceae

member. or Compositae spouses, is also known as sweat herb and is becoming increasingly important as a natural sweetener. It has a high concentration of stevioside and rebaudioside, two types of herbal sugar. Another name for it is honey leaf. This plant is indigenous to South America. Since it was anticipated to be 0.843 million tonnes, the difference between Egypt's sugar output and use (2.6 metric tonnes) of (1.757 metric kilogrammes) constitutes a significant concern. This issue was resolved by introducing stevia to the area. Stevia use in the food industry is the focus of attention these days in an effort to reduce the difference between consumption and production [2]. Human intestine bacterial microflora may break down



stevioside into steviol, which is its main metabolite [6–8]. Glycoside steviol, which is extracted stems of *Stevia rebaudiana* and has a sweetening power 250–300 times more than sucrose, is also used to treat metabolic illnesses related to the metabolism of carbohydrates, such as overweight and obesity, diabetes mellitus, hypertension, and other conditions and it also promotes cell renewal [1]. The primary and essential sweetening chemicals found in stevia extracts are the a substance called (The CAS programme number. 57817-89-7) & rebaudioside-A (the course no. 58543-16-1) glycoside that's chemical compounds, which are present in higher percentages in these extracts than in lower proportions in other steviol glycosides. The primary factors affecting the composition of leaf extracts are the extraction and purification techniques employed, as well as the composition of the leaves themselves, which is impacted by soil and climate [7]. According to toxicological research, stevioside does not cause mutagenesis, teratogenicity, or cancer. It has also not been linked to any adverse responses when employed as a sugar substitute. Therefore, The plant sweetener and its a compound called Used for diabetic treatment mellitus, obesity, high arterial pressure, and preventative in place of sugar. [2] . Its antibacterial, antimicrobial, anti-retoviral, anti-oxidant, and hypoglycemic properties have all been noted and documented. Because of its rejuvenating nature, it has a remarkable ability to minimise premature ageing of the skin. It is extensively employed in the production of cosmetics and other beauty items. As a result, they are also extensively used in the manufacture of wine, food, beverages, medicines, and skincare., etc. Its bitter taste restricts its suitability for human ingestion. Rebaudioside A, a stevioside, is the primary ingredient in stevia extract. The global trend towards using natural ingredients in food and cosmetics has led to a sharp increase in interest in natural antioxidants. Plant-based natural antioxidants, particularly phenolics and flavanoids, have shown significant promise in medicine and are being used in the market as dietary supplements or antioxidant additives [13–15]. The purpose of this work is to isolate stevioside from stevia leaves using inexpensive techniques and standard laboratory supplies.

2. Objectives

. An attempt was made to use low-cost, easily scaled, and straightforward methods, such as TLC Chromatography and soxhlet extraction, to extract and purify the sweetening ingredient from Bertoni *Stevia rebaudiana*. The current work developed a new, enhanced method for removing sweetening ingredient from stevia leaves utilising a standard solvent extraction procedure. To address the enormous demand for natural sweeteners, this technology can be expanded at the industry level for large-scale production in the future.

3. Methods

3.1. Preparation of Stevia Extract

After being pulverised, 100g of stevia leaves were extracted with one litre of water and after four hours of stirring 600 c. To extract all of the stevioside, the procedure was repeated using the leftover material from the initial extraction. To get the residue, the two filters were concentrated together by distillation using a rotary evaporator.

3.2. Isolation of Stevioside from the Residue

To separate the stevioside from the water and transfer it into the butanol using a separating funnel, the residue needs to be rinsed three times in butanol.

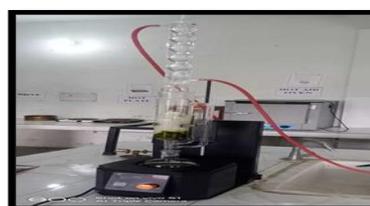


Figure-1 Isolation of Stevioside



Figure-2 Pharmacognostic and phytochemical analysis of stevioside stevia leaf extract



3.3 Thin Layer Chromatography of Isolated Stevioside

Water, a solution of and the acetate ethyl (7.5: 1.5:10) were the mobile phase used in the Thin Layer Chromatography (TLC) procedure. Using a detecting agent, such as conc.H₂SO₄: Ethyl alcohol (1: 10), the spot was visualised.

4. Results

TABLE 1 - Result of TLC

S. no	Compound	Mobile phase(mp)	Detecting agent(da)	Rf values(cm)
1	Stevioside (solute A)	Ethyl-acetate: Methanol: Conc.H ₂ SO ₄ (7.5:1.5:10)	Conc.H ₂ SO ₄ : ethyl alcohol (1 : 10)	0.50
2	stevioside (solute B)	Ethyl-acetate: Methanol: Conc.H ₂ SO ₄ =(7.5:1.5:10)	Conc.H ₂ SO ₄ : ethyl alcohol=(1 : 10)	0.67



Figure-3 TLC Paper

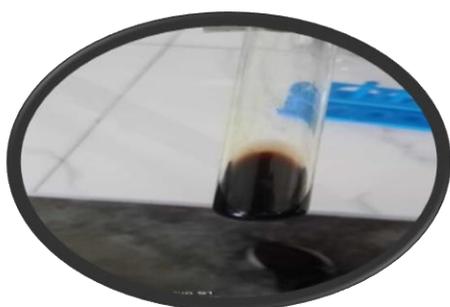


Figure-4 crystallization of stevioside



Figure-5 Identification of Stevioside

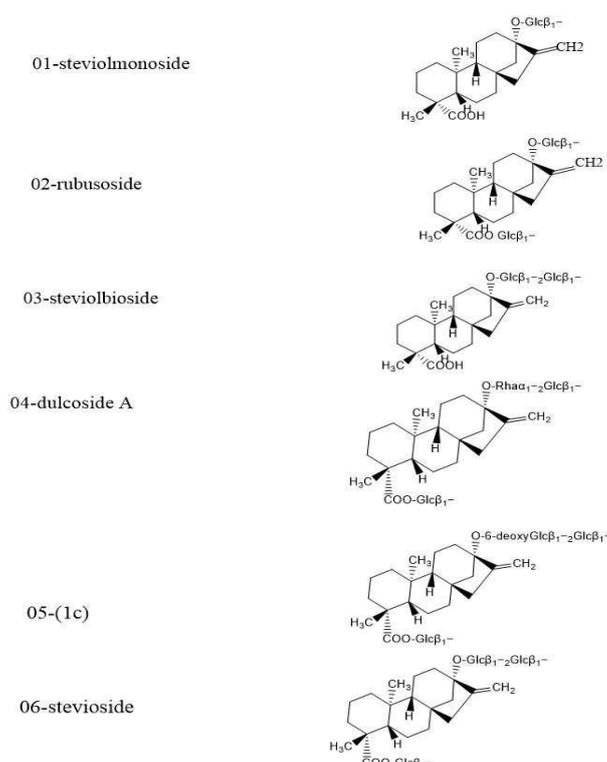


Figure-5 Structure of Stevioside and Chemical Formula: C₃₈ H₆₀ O₁₈



Figure-6 Pharmacological and Pharmacognostic application of Stevioside



Figure-7Traditional Application of Stevioside

5. Discussion

Using two distinct solvents with Rf values of 0.50 and 0.67 on TLC, stevioside was effectively separated. It was then further purified and recrystallized. The low-calorie steviol glycosides called steviosides are found in its *Rebaudiana Bertoni* the plant's leaflets. They are frequently used by diabetic people as a natural sweetener. The stevioside band is visible in the TLC data, and this is verified by contrasting the samples' Rf value (0.50) with the standard's.

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