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Application of natural sweetener *Stevia rebaudiana* in the medical field

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ABSTRACT. Steviol glycoside sweeteners have been isolated and produced for hundreds of years in Bertoni, a South American Asteraceae plant prized for its sweetness. Stevia is becoming more popular among consumers and food manufacturers as a result of diabetes, rising obesity rates, and other related comorbidities, as well as global public policies calling for a reduction in intake of sugar to help control these issues. Low-calorie as well as no-calorie sweeteners, such as Stevia, have this appeal because they are plant-based, have no calories, and have a sugary flavour that is 50 to 350 times sweeter than table sugar, making them an excellent as well as suitable choice for use in foods as well as beverages with lower sugar and calorie content. This plant yields steviol glycoside sweeteners, which are isolated and processed. Current studies on *Stevia rebaudiana* extract, metabolism, tolerable intake, and safety, steviol glycoside extraction and purification, side effects, as well as the toxicity of *Stevia* extract are discussed in detail.

Keywords: sweetener; steviol glycosides; diabetes; obesity; metabolic activity; *S. rebaudiana*.

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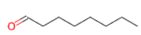
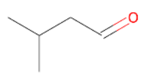
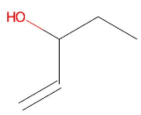
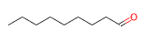
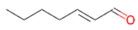
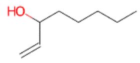
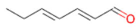
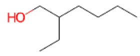
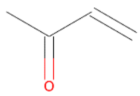
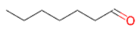
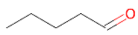
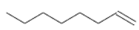
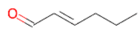
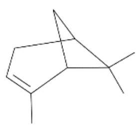
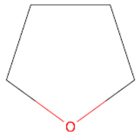
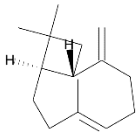
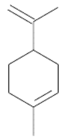
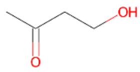
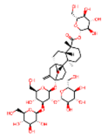
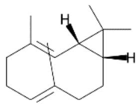
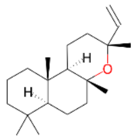
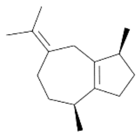
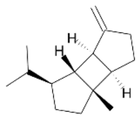
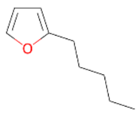
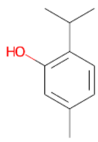
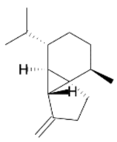
Introduction

Stevia is a sweet herb with the scientific name *Stevia rebaudiana* Bertoni from the kingdom Plantae, family Asteraceae and genus *Stevia*. It is also referred to as the 'sweet herb of Paraguay' since it is sugary by nature and is a native of that country. Other names for it include honey leaf, candy leaf, sweet leaf, sweet herb, and honey. The leaves are an exquisite shade of green and are flavorful. 'stevioside' and *rebaudioside* are the two chemicals found in the *Stevia* leaves, and are 200 times sweeter than sugar (Abou-Arab, Abou-Arab, & Abu-Salem, 2010). The different phytochemicals found in *Stevia rebaudiana* are summarized in Table 1 with their molecular formula, molecular weight, and structure.

Table 1. *Stevia rebaudiana* phytochemicals (Mohanraj et al., 2018; Vivek-Ananth, Mohanraj, Sahoo, & Samal, 2023).

Sr. No.	Phytochemical Name of compound	Molecular Formula	Molecular Weight (g mol^{-1})	2-D Structure
1.	Octanal	$C_8H_{16}O$	128.22	
2.	3-Methylbutanal	$C_5H_{10}O$	86.13	
3.	1-Penten-3-OL	$C_5H_{10}O$	86.13	
4.	Nonanal	$C_9H_{18}O$	142.24	

Sr. No.	Phytochemical Name of compound	Molecular Formula	Molecular Weight (g mol^{-1})	2-D Structure
5.	2-Heptenal	$C_7H_{12}O$	112.17	
6.	1-Octen-3-OL	$C_8H_{16}O$	128.22	
7.	2,4-Heptadienal	$C_7H_{10}O$	110.16	
8.	2-Ethylhexanol	$C_8H_{18}O$	130.23	
9.	Hexanal	$C_6H_{12}O$	100.16	
10.	Decanal	$C_{10}H_{20}O$	156.27	
11.	Methyl vinyl ketone	C_4H_6O	70.09	
12.	Heptanal	$C_7H_{14}O$	114.19	
13.	Pentanal	$C_5H_{10}O$	86.13	
14.	1-Octene	C_8H_{16}	112.22	
15.	1-Pentanol	$C_5H_{12}O$	88.15	
16.	2-Hexenal	$C_6H_{10}O$	98.15	
17.	alpha-Pinene	$C_{10}H_{16}$	136.24	

Sr. No.	Phytochemical Name of compound	Molecular Formula	Molecular Weight (g mol^{-1})	2-D Structure
18.	Tetrahydrofuran	C_4H_8O	72.11	
19.	beta-Caryophyllene	$C_{15}H_{24}$	204.36	
20.	Limonene	$C_{10}H_{16}$	136.24	
21.	1-Heptene	C_7H_{14}	98.19	
22.	4-Hydroxy-2-butanone	$C_4H_8O_2$	88.11	
23.	Rebaudioside A	$C_{44}H_{70}O_{23}$	967.02	
24.	Bicyclogermacrene	$C_{15}H_{24}$	204.36	
25.	Labd-14-ene, 8,13-epoxy-, (13S)-	$C_{20}H_{34}O$	290.49	
26.	beta-Guaiene	$C_{15}H_{24}$	204.36	
27.	(-)-beta-Bourbonene	$C_{15}H_{24}$	204.36	
28.	2-Pentylfuran	$C_9H_{14}O$	138.21	
29.	Thymol	$C_{10}H_{14}O$	150.22	
30.	beta-Cubebene	$C_{15}H_{24}$	204.36	

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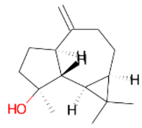
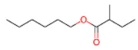
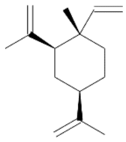
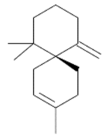
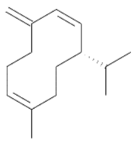
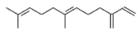
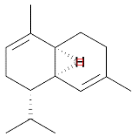
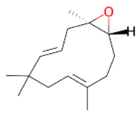
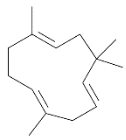
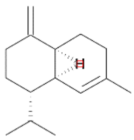
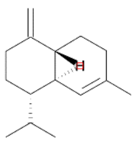
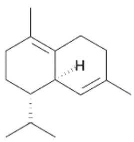
31.	Spathulenol	$C_{15}H_{24}O$	220.36	
32.	Hexyl 2-methylbutanoate	$C_{11}H_{22}O_2$	186.3	
33.	beta-Elemene	$C_{15}H_{24}$	204.36	
34.	(-)-beta-Chamigrene	$C_{15}H_{24}$	204.36	
35.	1-Nonene	C_9H_{18}	126.24	
36.	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	$C_{15}H_{24}$	204.36	
37.	beta-Farnesene	$C_{15}H_{24}$	204.36	
38.	alpha-Muurolene	$C_{15}H_{24}$	204.36	
39.	Humulene epoxide II	$C_{15}H_{24}O$	220.36	
40.	Humulene	$C_{15}H_{24}$	204.36	
41.	gamma-Muurolene	$C_{15}H_{24}$	204.36	
42.	(+)-gamma-Cadinene	$C_{15}H_{24}$	204.36	
43.	(+)-delta-Cadinene	$C_{15}H_{24}$	204.36	

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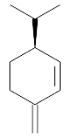
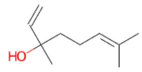
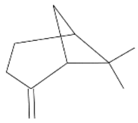
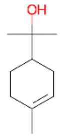
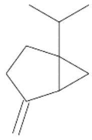
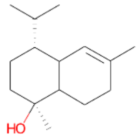
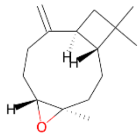
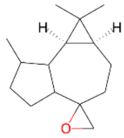
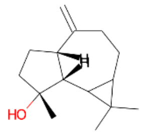
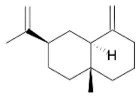
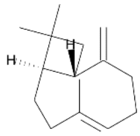
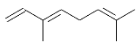
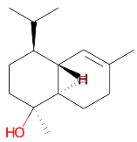
44.	(+)-beta-Phellandrene	$C_{10}H_{16}$	136.24	
45.	Linalool	$C_{10}H_{18}O$	154.25	
46.	beta-Pinene	$C_{10}H_{16}$	136.24	
47.	alpha-Terpineol	$C_{10}H_{18}O$	154.25	
48.	Sabinene	$C_{10}H_{16}$	136.24	
49.	(-)-alpha-Cadinol	$C_{15}H_{26}O$	222.37	
50.	Caryophyllene oxide	$C_{15}H_{24}O$	220.36	
51.	allo-Aromadendrene epoxide	$C_{15}H_{24}O$	220.36	
52.	(1aS,4aS,7R,7aS,7bS)-1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulen-7-ol	$C_{15}H_{24}O$	220.36	
53.	beta-Selinene	$C_{15}H_{24}$	204.36	
54.	beta-Caryophyllene	$C_{15}H_{24}$	204.36	
55.	(E)-beta-ocimene	$C_{10}H_{16}$	136.24	
56.	Cedrelanol	$C_{15}H_{26}O$	222.37	

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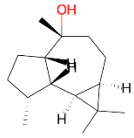
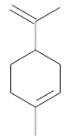
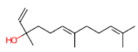
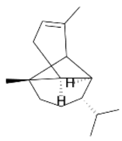
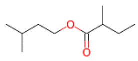
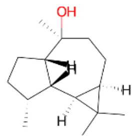
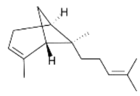
57.	Ledol	$C_{15}H_{26}O$	222.37	
58.	Limonene	$C_{10}H_{16}$	136.24	
59.	Nerolidol	$C_{15}H_{26}O$	222.37	
60.	alpha-Copaene	$C_{15}H_{24}$	204.36	
61.	3-Methylbutyl 2-methylbutanoate	$C_{10}H_{20}O_2$	172.27	
62.	Viridiflorol	$C_{15}H_{26}O$	222.37	
63.	trans-alpha-Bergamotene	$C_{15}H_{24}$	204.36	

Substituting sugars with artificial and natural sweeteners is a universally recognized strategy to combat obesity, hyperlipidemia, diabetes, and cavities. (Ahmed et al., 2010). For food, drinks, medications, dental hygiene, and cosmetic goods, sweetness is commonly considered a pleasant and desired flavor. The most commonly used natural energy-containing sugars to add sweetness to consumer goods are table sugar, fruit sugar, and dextrose (Priya, Gupta, & Srikanth, 2011). Customers who want a sugary flavor with low to no calories must go to alternative sources because these natural sugars carry calories (Prakash et al., 2014). Both natural and artificial sweeteners must be developed in such a way that they are cost-effective, patentable, and satisfy both needs of providing sweetness and no calories (DuBois & Prakash, 2012).

These developed sweeteners non-caloric or low-calorie sweeteners are difficult to develop because they differ from sugar in terms of temporal profile, maximum possible response, flavor pattern, taste, and adaption or behavior (Prakash, DuBois, Clos, Wilkens, & Fosdick, 2008). Extensive research on steviol glycosides extracted from *S. rebaudiana* Bertoni has been conducted to develop the ultimate sugar substitute (sugary, low-to-no calorie, and organic) (Chaturvedula & Prakash, 2011a; 2011b).

High-purity *rebaudioside M*, commonly known as *rebaudioside X*, is marketed by The Coca-Cola Company and Pure Circle Limited as a natural, calorie-free sweetener that is widely used in foods and beverages. Two novel diterpene glycosides were isolated after further purification of an extract made from the crushed leaves of *S. rebaudiana* (Chaturvedula, Rhea, Milanowski, Mocek, & Prakash, 2011). The two new glycosides were also reported as 13-[(2-O-(3-α-O-d-glucopyranosyl)-β-d-glucopyranosyl-3-O-β-d-glucopyranosyl-β-d-glucopyranosyl) oxy] ent-kaur-16-en-19-oic acid β-d-glucopyranosyl ester, and 13-[(2-O-β-d-glucopyranosyl-3-O-(4-O-α-d-glucopyranosyl)-β-d-glucopyranosyl-β-d-glucopyranosyl) oxy] ent-kaur-16-en-19-oic acid β-d-glucopyranosyl ester (Chaturvedula et al., 2011; Chaturvedula, Mani, & Prakash, 2011). Based on both 1D and 2D-NMR analyses, the structures of the aforementioned compounds were established (Ibrahim et al., 2014). One of the minor sweet ingredients in the South American herb *Stevia rebaudiana*

Bertoni is *rebaudioside M*. It is one of at least ten other sweet-tasting steviol glycosides found in nature. It is a glycoside of steviol, an ent-kaurene diterpenoid aglycone. *Stevia* sweeteners are legal in several countries, including the United States, the European Union, Japan, China, Brazil, and others (Prakash, Campbell, San Miguel, & Chaturvedula, 2012; Prakash, Campbell, & Chaturvedula 2012).

Stevia's alpha-glucose additions, which are altered via enzymatic transglycosylation, enhance flavor therefore it is popular in Japan. Numerous studies on the metabolism and excretion of *Stevia* combinations in mammals have shown that stevioside has no discernible harmful effects on mammalian species (Kinghorn, Kim, & Kim, 1999; Kinghorn, Wu, & Soejarto, 2001; Carakostas, Prakash, Kinghorn, Wu, & Soejarto, 2012). According to Koyama et al., human intestinal microflora completely hydrolyzed a *Stevia* combination made up of *rebaudioside A*, stevioside, and *rebaudioside C* into steviol in 24 hours. The intestinal metabolism of *Stevia* combinations in rats and humans did not appear to differ from one another. *Stevia* has thus been approved for usage as a sweetener (Kinghorn & Soejarto, 1985).

By carefully selecting offspring from *S. rebaudiana* Bertoni, a new cultivar, *S. rebaudiana Morita*, was created (Ohta et al., 2010). This cultivar is known to produce unusually high concentrations of *Rebaudioside A*, the sweetest steviol glycoside among the known stevioside (60 percent of the total). However, it was discovered that many unidentified glycosides were minor components in leaf extracts from *S. rebaudiana Morita*. Before *Stevia* sweeteners may be used as food additives, approximately 95% of their constituent chemicals must be identified (Martins, 2016).

The 'stevioside' and *rebaudioside A* having attracted significant commercial attention were reported as major two Steviol glycosides in *S. rebaudiana*. Apart from that these Steviol glycosides (1000 mg) did not interfere in glucose homeostasis (blood pressure) mechanism in individuals with Type II diabetes mellitus (Maki et al., 2008). Steviol biosides, dulcosides, and *rebaudioside C* are the additional glycosides that are found in lesser concentrations (Starratt, Kirby, Pocs, & Brandle, 2002).

Biological activities of *Stevia rebaudiana* extract

The two primary sweeteners in *Stevia* are 'stevioside' and *rebaudioside A*. In 1931, 'stevioside' was initially extracted from *Stevia*, and in 1952, its chemical composition was determined. Three molecules of glucone moiety and a glucose moiety, or steviol, make up the diterpene glycoside known as stevioside. 'stevioside' *s* account for 4 to 13% of all glycosides in *Stevia*. Apart from serving as a sweetner, *Stevia rebaudiana* also serves as an adjuvant in treating chronic diseases (Marcinek & Krejpcio, 2015).

Another steviol diterpene glycoside is *Rebaudioside A* with a sweetness percentage ranging between 30 and 40% and is 180 to 400 times sweeter than sugar (Kaplan & Turgut, 2019). 'stevioside' concentrations range between 6.5 and 9.1%, while *rebaudioside A* concentrations range between 2.3 and 3.8%. *Steviol monoside, rubusoside, dulcoside A*, and steviol bioside are all the most well-known substances that have been identified from *Stevia* leaves. Additionally, *Stevia* also provides nutritional benefits such as gross energy and high levels of protein in ruminant animals (Starratt et al., 2002; Savita, Sheela, Sunanda, Shankar, & Ramakrishna, 2004; Goyal, Samsher, & Goyal, 2010; Atteh et al., 2011).

Dried *S. rebaudiana* leaves contain 0.2 percent and 0.1 percent of *Rebaudioside D* and 0.1 percent of *rebaudioside M*, respectively (Neuwirth, 2020). The Coca-Cola Company, on the other hand, continues to market *rebaudioside M* for use in foods and drinks (Prakash, Markosyan, & Bunders, 2014). *Rebaudioside D* can also be used as a non-caloric sweetening agent in the food industry. Steviol and isosteviol, produced as a byproduct of stevioside hydrolysis, have medicinal applications. The steviol glycoside extracts *Rebaudioside A* and *Rebaudioside D* from *Stevia* vary in their bitterness at suprathreshold concentrations (Allen, McGeary, & Hayes, 2013).

Different sweet *Stevia* glycosides have different levels of sweetness and different amounts in the leaves. *Stevia* leaves that have been processed are typically 250 to 300 times sweeter than table sugar. The *Stevia* leaves contain proteins, carbs, lipids, oils, dietary fiber, phenolic compounds, and vitamins. Dried *Stevia* leaves have 11.2 to 16.0 g of protein, 61.9 g of carbs, 1.9 to 3.73 g of lipids, and 6.8 to 15.2 g of bulk per 100 g of dried mass. Additionally, the geographical region of the plant's growth affects its biochemical makeup (Khiraoui et al., 2017).

The presence of several phenolic compounds with potent antioxidant capabilities was discovered in *Stevia* leaves. The total polyphenol and flavonoid concentration in methanolic extracts was calculated to be 25, 18, and 21.73 mg g^{-1}, respectively. *Stevia* leaves are high in the water-soluble vitamins, vitamin C (14.98 mg 100 g^{-1}), riboflavin (0.43 mg 100 g^{-1}), and folate (52.18 mg 100 g^{-1}) (Kim, Yang, Lee, & Kang, 2011).

Metabolism of Steviol glycosides

Various scientific organizations and specialists, notably the European Food Safety Authority (EFSA) and most recently, Magnuson et al., have evaluated the absorption, digestion, and defecation of steviol glycosides in great detail. Steviol glycosides pass undigested through the upper gastrointestinal system. The steviol backbone is retained, absorbed systemically, glucuronidated in the liver, and eliminated through urine in humans and faeces in rats. They are only hydrolyzed or destroyed when they come into contact with bacteria in the colon that cleave the glycosidic bonds, eliminating the glycon (Magnuson, Carakostas, Moore, Poulos, & Renwick, 2016).

Studies conducted in vitro reveal that the glycosidic linkages found in ‘stevioside’ cannot be hydrolyzed by human saliva, salivary-amylase, pancreatin, pepsin, and pancreatic-amylase, as well as jejuna brush border enzyme from mice, rats, and hamsters. Therefore, ‘stevioside’ can be transformed into steviol by the gut microbiota of humans, rats, and hamsters (Hutapea, Toskulkao, Buddhasukh, Wilairat, & Glinsukon, 1997).

Reb-A and ‘stevioside’ were both entirely hydrolyzed to steviol in 10 and 24 hours, respectively, when they were incubated with human fecal bacteria. It is a zero-calorie sweetener since the released glycon is not absorbed and is likely used as an energy source by gut microorganisms soon. Steviol is not metabolized by gut bacteria and is instead absorbed from the colon, according to an in vitro model of the intestinal barrier, which has demonstrated that the transport of ‘Reb A’ and ‘stevioside’ through the monolayers is very low, whereas the transport of steviol is very high. Through the action of their glycosidase, bacteroides species are principally in charge of the hydrolysis of steviol glycosides in the stomach (Gardana, Simonetti, Canzi, Zanchi, & Pietta, 2003).

Characteristics of extracted ‘stevioside’ and *Rebaudioside* A from *Stevia* leaves

The active ingredients are the steviol glycosides (‘stevioside’ and *rebaudioside*), that are heat as well as pH-resistant, 30 to 150 times sweeter than table sugar, and not fermentable. The excessive consumption of sugar is becoming increasingly risky because it leads to numerous health issues, particularly diabetes. To protect the health of customers, a replacement sweetener must be created. In the recovery process known as solid-liquid extraction, specific components of a solid material are recovered by the extracting solvent. Steviol glycosides from *Stevia* plants have been extracted using a variety of techniques. One of these entails environmentally friendly glucose extraction using ethanol and water (Muthusamy & Munaim, 2019).

Table sugar is typically used as the primary sweetening agent in the food sector. Customers are becoming more interested in natural-source sweeteners based on *Stevia*. Additionally, the sweetener is risk-free for consumption, has nearly few calories, is unaffected by heat and pH changes, and tastes just like sugar. The daisy family includes the herb *Stevia*, which has a pleasant flavor.

Steviol glycosides' security and recommended daily intake

All major international scientific and legal bodies have determined that elevated steviol glycosides are fit for human consumption. The vast majority (95 percent) of regulatory approvals are for increased steviol glycosides. When compared to unprocessed, crude *Stevia* extracts, well-characterized, high-purity steviol glycosides have not been shown to have any negative effects on reproduction in animals when used in food and beverages. As a result, qualified science-based professionals and governing bodies have determined that crude extract studies are inapplicable to the safety assessment of rising steviol glycosides (Perrier, Mihaloy, & Carlson, 2018).

Rats have been used as the primary animal model for studying the potential future effects of increased steviol glycosides on chronic and acute toxic effects, reproductive problems toxicity, and carcinogenic effects. Although the final route of excretion differs, a significant proportion of the body's cells and tissues are exposed to the same metabolites at comparable concentrations for a comparable span of years after consuming steviol glycosides, including both organisms, implying that the possibilities for toxicological effects are similar (Melis, 1999).

Extraction and Steviol glycosides purification

The production of steviol glycosides must extract and purify them from plant material in a way that yields a high purity of unaltered molecules. Dehydration or drying of plant material is required as a first step to prevent microbial development and biochemical changes. Common dehydration techniques include freeze drying, convection drying, vacuuming, microwave drying, infrared drying, sun drying, and shadow drying. The drying conditions used have a significant impact on the amount of total and specific steviol glycosides extracted from fresh *Stevia* leaves. Dehydration or drying of plant material is required as a first step to prevent microbial development and biochemical changes. Shade drying has been claimed to be the least harsh treatment for all steviol glycosides (Periche, Castelló, Heredia, & Escriche, 2015).

However, drying in the shade increases the risk of contamination, which reduces the quality of the organic material. A variety of traditional and cutting-edge technologies are used to extract steviol glycosides, including heat extraction and maceration, high pressure and high temperature, radiation, chromatographic, electrical voltage, and ultrasound methods. Depending on the solvent and method used, the extraction yields obtained using these technologies range between 2 and 35 percent. Applying these technologies to large-scale production is difficult due to the low percentages and vast volumes of organic solvents that are eliminated later in the refining process. Fibroblast technologies are now being proposed as one of the efficient methods of extracting Steviol glycosides. These techniques, which include microfiltration, ultrafiltration, and nanofiltration, are still in the early stages of development (Díaz-Montes, Gutiérrez-Macías, Orozco-Álvarez, & Castro-Muñoz, 2020). The bioactivities and extraction methods of *stevia* along with their structure are summarized in Figure 1.

Semi-permeable membranes are commonly used in pressure-driven membrane processes as a protective layer for both the movement of chemicals inside a solution and the Membrane processes have many advantages, including low energy consumption, shorter extraction times, improved dispersibility, adaptability, productivity increases, and ease of leveling. Furthermore, because successful extraction requires only a small amount of chemical solvent, membrane techniques are friendly to the environment. Steviol glycosides are particularly interested in this because of their intended use in the pharmaceutical and food industries. According to the most recent data, the removal efficiency of 'stevioside' and *rebaudioside A*, as well as their cleanness, are now in the spectrum of 19 to 90 percent and 32 to 98 percent, respectively, when using integrated membrane methods.

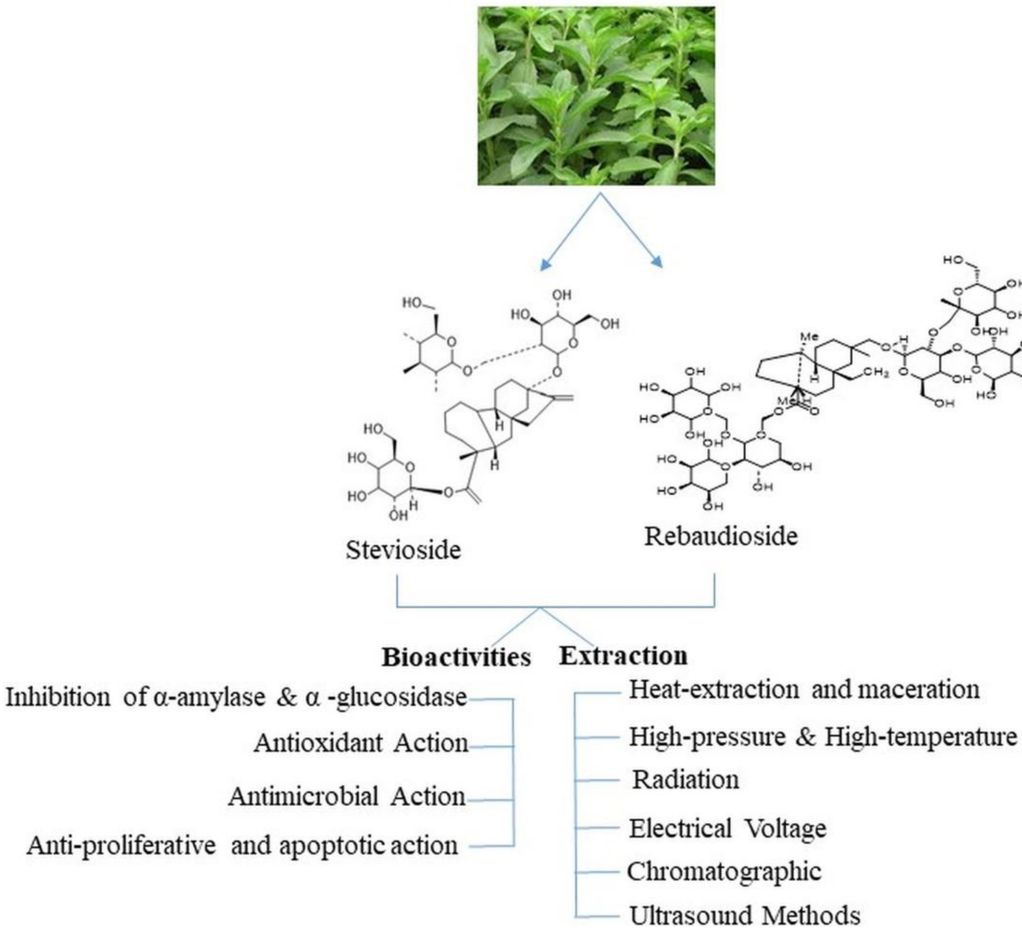


Figure 1. Bioactivities and extraction methods of *Stevia*.

The value of the extraction yield is affected by several variables, including operational factors, inherent membrane characteristics, and pretreatment procedures. Last but not least, it's critical to take into account the potential for using a 'green' solvent to maximize the extraction of steviol glycosides. These solvents are distinguished by minimal toxicity, simple accessibility, ease of reusability, and great efficacy. Additionally, the development of 'green' solvents for the extraction of significant trade chemicals is becoming an increasingly essential area of research due to legislative considerations and the changing attitude toward environmental issues. Although it seems that water, an all-purpose solvent, is sufficient for the extraction of steviol glycosides, it cannot be completely ruled out that the recovery of these compounds can benefit from the use of other 'green' solvents (Castro-Muñoz et al., 2022).

Toxicity and side effects

The European Food Safety Authority's Scientific Committee and the Food and Drug Administration have established 4 mg kg^{-1} body mass as the appropriate daily dose of Stevia dry extract. One animal study found that *Stevia* preparations were allergenic. Because crude extracts are much more likely to contain allergic chemicals found naturally in the Asteraceae family, they may be more allergenic than ultrapure *Stevia*-based sweeteners containing 95 percent steviol glycosides (Urban, Carakostas, & Taylor, 2015).

Previous research found that *Stevia* extract reduced rat fecundity by about 21% when compared with a control group of rats. After only a 50 to 60 day recovery period, fertility was still down by 47 percent. *Stevia* administration resulted in a significant decrease in the number of spermatozoa stored, as well as a decline in the weighting factor of the prostatic urethra and testis (Melis, 1999).

Steviol is mutagenic, according to one study, although this impact has not been supported by other research. The main proof of *Stevia's* safety is the absence of any reports of side effects in over 1500 years of continuous consumption by Paraguayans. The absence of any complaints of negative effects of any type among Japanese communities, where *Stevia* has been consumed extensively in recent years, provides more evidence of the safety of *Stevia* usage. Additionally, none of the research that looked at how *Stevia* affected people's bodies found any negative effects (Ucar, Ozyigit, Demirbas, Guven, & Turgut, 2017). It should be noted, nonetheless, that not all Stevia products on the market are of premium quality. In one experiment, the Ra-man spectroscopy of six productions of *Stevia* goods was evaluated, and three of what looked like the advertisement *Stevia* products were discovered to be forgeries. There were also trace amounts of sodium saccharin and sodium cyclamate (Peteliuk, Rybchuk, Bayliak, Storey, & Lushchak, 2021).

Application of *Stevia* in the healthcare sector

Diabetes treatment with Stevia

Diabetes mellitus is a group of diseases wherein hormones are either ineffectively used or insufficiently produced (type 1 diabetes mellitus; type 2 diabetes mellitus). As of 2019, diabetes affects 464 million people worldwide. In 2000, 175.4 million people were diagnosed with the disease. As per the International Diabetes Federation, 642 million people worldwide will have diabetes by 2040. The disease is diagnosed in approximately 90% of patients in both emerging and developed countries (Vaiserman & Lushchak, 2019).

The most common causes of type 2 diabetes mellitus are biological factors, poor nutrition, and a lack of physical exercise. Type 2 diabetes mellitus is caused by impaired insulin metabolic activities in the digestive system and elevated insulin levels in extracellular fluid that have decreased reactivity to increasing insulin concentration levels and are unable to use blood sugar (Vaiserman & Lushchak, 2019).

In Brazil and Paraguay, traditional medicine has long used *Stevia* not just as a sweetener but also as a medication to treat diabetes and hyperglycemia. According to one study, diabetic rats given an aqueous leaf extract of candy leaves had higher insulin and carbohydrate levels than the comparison group. Furthermore, rebaudioside hormone production increased after isolated mouse Langerhans cells were treated. Furthermore, 'stevioside' also improves hormone sensitivity; in particular, small doses of the compound increase hormone impact on gluconeogenesis in muscle tissue (Ahmad & Ahmad, 2018).

According to other research, *Stevia* and Steviol could be effective in the treatment of diabetes mellitus because they act as general pro-drugs by influencing pancreatic beta cells, and their insulinotropic activity is terminated when blood glucose levels fully recover. At normal glucose levels, the absence of insulin-stimulating effects may reduce the chance of low blood sugar. When compared to a control group, diabetic mouse groups that consumed the antioxidant portion of *Stevia* nutrients demonstrated improved glucose tolerance (Urban et al., 2015).

Stevia's anti-hypertension properties

High blood pressure, also known as hypertension, is still the most dangerous indicator of the risk of heart attack and stroke. It may be a distinct illness or a symptom of other conditions such as natural abnormalities of the brain, atherosclerosis, chronic renal and endocrine organ illnesses, and some cardiac problems (Oparil et al., 2018).

In one experiment, rats with spontaneous hypertension were used to examine the impact of intravenous stevioside on blood pressure (SHR). In conscious SHR, intravenous dosages of 50, 100, and 200 mg kg^{-1} had a dose-dependent hypotensive impact on both systolic and diastolic blood pressure. With a dosage of 200 mg kg^{-1}, the hypotensive effect persisted for more than 60 min. Stevioside was therefore successful in lowering blood pressure (Chan et al., 1998). The effect of *Stevia* compounds in lowering blood pressure in Cardio Vascular Disease (CVD) has been shown in Figure 2.

Stevia effects on obesity

Globally, the percentage of obese and overweight people is increasing, and obesity is now recognized as a serious global health issue. Obesity, on the other hand, is a complicated condition with diverse physiological issues, such as insulin resistance, leukemia, heart disease, stroke, and sleep apnea. It is more than just being overweight. Obesity significantly increases the risk of various illnesses, resulting in a lower quality of life for people. Abdominal obesity is among the components of diabetes mellitus, which increases the risk of heart disease (Bayliak, Abrat, Storey, Storey, & Lushchak. 2019). This includes dyslipidemia and hepatic steatosis. According to research, consuming sugary syrups can help reduce body fat by lowering caloric intake.

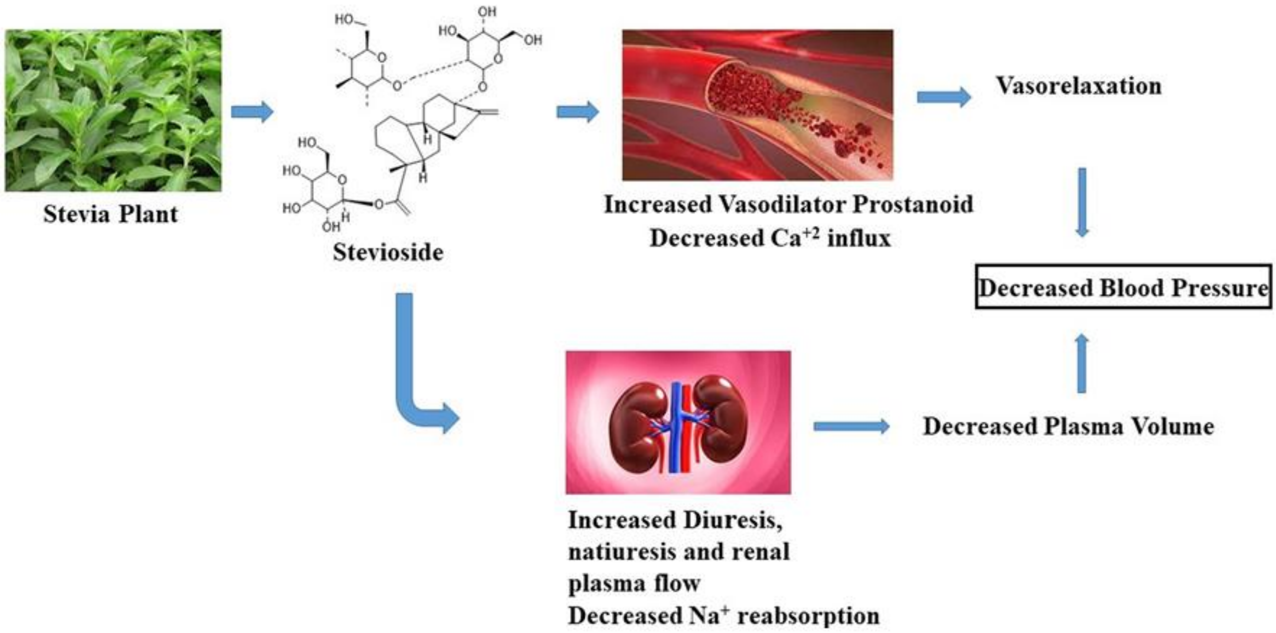


Figure 2. Effect of *Stevia* compounds in Cardiovascular Disease (CVD) management.

Consumption of *Stevia* sweetener also enhanced high-density lipoprotein levels and decreased total cholesterol, triglycerides, and low-density lipoprotein concentrations in the blood of experimental rats (Ranjbar et al, 2020). Supplementing with *S. rebaudiana* extract reduced serum and liver triacylglyceride levels throughout the mouse model that consumed an increased diet. The treated mice were then supplied with an increased diet without *Stevia* extract. Furthermore, mice fed diets containing food supplemented with *Stevia* extract had higher levels of carnitine, a substance important for lipid metabolism because it transports lipids from the cell cytoplasm to the mitochondria, in which they are oxidized. Another human study discovered that the major difference in total calories between the *Stevia* extract consuming control conditions was due solely to calorie intake change and that *Stevia* extract use had no effect on hunger or appetite.

Increased inflammation, oxidative stress, and insulin resistance are all frequently linked to obesity. According to one study, stevioside treatment in obese insulin-resistant mice improved insulin signaling and antioxidant defense, which in turn inhibited the development of atherosclerotic plaque. Furthermore, *Stevia* extract has been shown to protect obese rats from the negative effects of increased high-sucrose nutrition on total cholesterol, phenolic acids overall, and histological variables (Farhat, Berset, & Moore, 2019).

Conclusion

Consumption of *Stevia* sweetener increases high-density lipoprotein levels and vice-versa it decreases the bad cholesterol levels, lipids, and poor glycoprotein concentrations in the blood. Genetic predisposition, an unhealthy diet, and insufficient physical activity are the main causes of the onset of type 2 diabetes. High blood pressure, also known as hypertension, is still the most dangerous predictor of heart attack and stroke risk. In one study, rats with spontaneous hypertension were used to see how intravenous stevioside impacted blood pressure (SHR). In conscious SHR, intravenous dosages of 50, 100, and 200 mg kg^{-1} had a dose-dependent hypotensive effect on both systolic and diastolic blood pressure. The hypotensive effect of 200 mg kg^{-1} lasted for more than 60 min. 'stevioside' was thus effective in lowering pressure. On the other hand, obesity is a complex condition with numerous physiological issues, which include diabetes mellitus, leukemia, cardiovascular disease, stroke, and sleep disorders. *Stevia* extract intake in control conditions was due solely to a change in calorie intake, and *Stevia* extract use did not affect hunger or appetite.

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