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Stevia efficacy as a natural sweetener

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Abstract
Stevia rebaudiana, is also known as sugar leaf or candy leaf. It is an ancient South American perennial shrub, belonging to the Asteraceae (Compositae) family that has been valued as a low-and-no-calorie-sweetener (LNCS). Since it is a natural sweetener, devoid of calories that is 50-350 times more sweet than sugar, stevia leaf extract is gaining popularity among health-conscious customers throughout the world. Sweet-tasting compounds called steviol glycosides are abundant in stevia leaf extracts. In addition to their sweet taste, these molecules have been attributed with therapeutic properties like antioxidant, antibacterial, and antifungal activity. The glycosides in stevia are not metabolised by the human body, hence it contains zero calories. The global public health guidelines urge for a reduction in the intake of sugar to help curb the rising incidents of diabetes and obesity. Sucralose is an artificial noncaloric sweetener, whereas stevia is a natural non-caloric sweetener. The key factors implicated in obesity-related comorbid disorders include insulin resistance - as in type 2 diabetes, elevated inflammation, that may pose a major risk factor conducive for developing a metabolic syndrome, such as CVDs, diabetes, and cancer, and oxidative stress i.e. depleted antioxidant sources. Extensive initiatives to successfully combat the surging tide of obesity, such as behavioural adjustments, anti-obesity treatments, and invasive surgical procedures, have met with little success. As a result, novel approaches to combating obesity, diabetes, and related illnesses are urgently required. This review aims to investigate where stevia research currently stands, as well as its efficacy, dependability, and potential health benefits, and where the herb may be headed in the future.

Keywords: Anti-diabetic, Anti-hypertensive, Anti-inflammatory, chelation, gut microbes, health benefits, low calorie, metabolic syndrome, obesity, oxidative damage, proliferating tumor, pure steviol glycosides

Introduction
Stevia rebaudiana, a natural calorie-free sugar substitute (Singh and Rao, 2005) [56], has attracted substantial attention from the research scientists owing to its impact on metabolic homeostasis, blood pressure regulation, and control of inflammatory processes, all of which are well-known obesity-related outcomes. Stevia leaf preparations can be considered to be safe for individuals with hypertension, type 2 diabetes, and obesity rather it is considered beneficial to treat or avoid complications of these diseases (Carrera-Lanestosa *et al*., 2017) [8]. The active compounds (steviol glycosides) have been shown to be safe to be consumed by children according to Aguero and co-workers, (2014) [1]. The principal constituents that provide the sweetness are Stevioside and rebaudiosides (Steviol diterpene glycosides). They are suitable for use in cooked foods because they can resist temperatures up to 200 degrees Celsius. The main sweetening substances of significance are *Stevioside* and *rebaudioside* A. Stevia may play a pivotal role as a potential remedy for the primary cardiovascular risk indicators linked with being overweight. Additionally, stevia has a number of other bioactive compounds that are potentially beneficial for human health (Ray *et al*., 2020) [50]. Because consumer demand for herbal foods is booming, *S. rebaudiana* has enormous potential as a new agricultural crop. Individuals who follow a low-carbohydrate diet would benefit greatly from the increased growth and manufacturing of stevia, which allows for a sweet flavour with less calories. Several global food regulatory and safety bodies have confirmed stevia's safety, but there is still a lack of information about the substance and the advantages it may provide.

The food and beverages sectors are aggressively proceeding towards producing low-calorie, low-sugar consumable items for consumers to avoid obesity and metabolic syndrome.

Many commercially available, low-calorie, chemical-based sweeteners have been proposed as sugar replacements in foods and drinks. Sucralose, cyclamate aspartame, xylitol, saccharin, acesulfame potassium, and neotame are some of the sweetening-additives linked to improved metabolic-and-health benefits, targeting obese individuals as well as diabetics, as the most vulnerable consumers (Azimi-Nezhad and collaborators, 2008; Anton, 2010; Lozano, 2010) [5, 2, 31]. Despite being deemed harmless, these sugar substitutes are not nutritious and are the topic of debate because they comprise chemical-based synthetic sweeteners. However, according to investigations by Tandel (2011) and Swithers, (2013) [71, 72], artificial sugar substitutes promote being overweight and thereby raise the likelihood of disorders of metabolism, heart ailments (CVD), and diabetes (type 2). In order to sustain the sensitivity of insulin and type 2 diabetes, natural sweeteners like stevia (sourced from the leaves of *Stevia rebaudiana*) are gaining popularity as herbal substitutes for synthetic sweeteners and sugars.

Stevia leaves contain over 30 different Steviol diterpene glycosides. The principal constituents that provide the sweetness are according to Marcinek and Krejpcio, (2015) [35], stevioside (4%-13% of all glycosides, 150-300 times more sweet than sugar, and according to Kaplan and Turgut, (2019) [28], *rebaudioside A* (30%-40% of all glycosides, 180-400 times more sweet than sugar). According to Ohtani and co-workers, (1992) and Uhler and Yang, (2018) [73] many different glycosides are reported in the sweet leaves of the sweet tea plant of *Rubus suavissimus.*

Background on *Stevia*

Botanist Moises Santiago Bertoni is credited for bringing stevia to the public's attention in the year 1901, as a plant with leaves that could sweeten a big cup of tea (Ramesh *et al*., 2006) [49]. In 1931, Briedel and Lavieille, two French scientists, found the glycoside that gives Stevia its sweet taste. Barriocanal and coworkers, (2008) [6] called this chemical stevioside. During World War II, there was a lack of sugar and it was rationed in the UK (Zaman *et al*., 2015) [69]. This made people start to use Stevia more.

Japan is among the world's leading Stevia producers (Ramesh *et al*., 2006) [49]. Drinks sweetened with Stevia as an alternative to sugar containing 30% fewer calories were introduced by the Coca-Cola Company in 2013. Many different countries throughout the world currently stock these beverages (Heyden, 2013) [76].

According to a 2014 study by Prakash and co-workers, [46] *rebaudioside M* is being marketed by The Coca-Cola Company for use in beverages and food products.

Certain food industries use *Rebaudioside D* as a no-calorie sweetener (Allen *et al*., 2013) [74].

The chemical contents and extent of sweetness of the different glycosides are different within leaves. The sweetest constituent glycosides of *Stevia* are Stevioside and rebaudioside (Marcinek and Krejpcio, 2015) [35].

Where the plant was grown has an effect on its biological composition (Khiraoui *et al*., 2017) [27]. Dried leaf preparations have a different chemical makeup than fresh leaf preparations, and this difference is further influenced by the processing or extraction method used (Snehal and Madhukar, 2012) [57]. It has been discovered that Stevia leaves contain many phenolic compounds with high antioxidant potential (Tadhani *et al*., 2007) [75].

Processing of Stevia

Analogous to how sugar cane is processed into sucrose, stevia leaves must undergo a series of extraction and purification steps before they can be utilised to make high-purity stevia.

In order to begin the process of extracting and purifying steviol glycosides, the leaves of the *S. rebaudiana* are picked, air dried, and pulverised (EU Commission regulation, 2012; FAO/WHO, JECFA. 2010) [13, 14]. They are then prepared as a tea decoction by being soaked in hot water (US FDA, 2008) [62]. The monosaccharide constituent of steviol glycosides renders them soluble in water, facilitating for their efficient extraction in industrial manufacturing facilities. Additionally, other components that constitute the leaves, such as proteins, fibres, pigments, polyphenolic substances, mineral substances, and salts, are also removed, giving the resulting water a dark brown colouration. The non-sugar components are eliminated throughout the extraction process, and the spray-dried pearl-white intermediary that contains 80-95% steviol glycosides is the ensuing outcome, according to Oehme and associates, (2017) [39]. Further, the intermediary product is crystallised in aqueous or ethanolic solutions, resulting in a white solid with a purity index of > 95%. Concentrating steviol glycosides necessitates a physical purifying technique that removes impurities from the leaves (Ashwell M, 2015) [4]. The molecular structure and pharmacological integrity of the steviol glycosides is preserved throughout the extracting as well as the purifying process, just as it occurs within the leaves (Oehme *et al*., 2017) [39].

Health benefits of Stevia

Stevia has been investigated for over a century. Its leaf preparations have been shown to be safe for human consumption in both historical and contemporary investigations, and their health advantages continue to be uncovered (Peteliuk *et al*., 2021) [44]. Given that there is a growing market for natural products and since close investigation has revealed that *Stevia* additionally includes folic acid, vitamin C, along with all of the essential amino acids (with the possible exception of tryptophan), giving it considerable opportunity as a new agricultural product in a market where demand for herbal products is rising. Those on a low-carbohydrate diet would benefit greatly from the increased growth as well as production of stevia, which allows for a sweet flavour with less calories.

The medicinal properties of compounds obtained from Stevia include:

1. The Anti-diabetic property: Diabetes is a kind of medical condition wherein either insulin production is inadequate (type 1 diabetes) or insulin resistance prevents it from being utilised properly (type 2 diabetes). While the number of people diagnosed with diabetes were 175.4 million in 2000, the number increased to 464 million people in 2019. By 2040, the number of people with diabetes is expected to reach 642 million according to Zimmet and associates, 2016 [70], (as reported by the International Diabetes Federation). Diagnosis of type 2 diabetes is around 90% worldwide, according to Tao and associates, (2015) [59] and Vaiserman and Lushchak, (2019) [65-66].

   Type 2 diabetes is largely caused by a combination of ancestral vulnerability, poor food habits, and insufficient exercise. Challenged digestion of carbohydrates in the hepatic system and insulin insensitivity in peripheral organs, characterised by diminished response to normal insulin concentrations, defined the pathophysiology of type 2 diabetes. Therefore, despite the fact that the pancreas produces more insulin in people with type 2 diabetes, levels of glucose in the blood rise (Vaiserman & Lushchak, 2019) [65-66].

Traditional Brazilians and Paraguayan medical practitioners have used Stevia for decades for the management of hyperglycemia and diabetes (Chand and Kumar, 2016) [9]. Stevia extracts from leaves and their various constituents are currently being studied for their potential to treat diabetes.

2. Anti-hypertensive property: Heart attack and stroke due to ischemia (reduced blood flow) are both greatly enhanced by hypertension, or high blood pressure. It can exist as a primary disorder or as a symptom of other disorders, including those affecting the kidneys and endocrine glands, the heart, the arteries, the brain, and the spinal cord (Oparil *et al*., 2018) [41].

   Capsules containing either 500 milligrams (mg) of stevioside powder or a dummy placebo were given to trial participants three times daily for two years. Oral stevioside considerably lowered systolic and diastolic blood pressure as opposed to dummy placebo among individuals with moderate hypertension, according to a study (Hsieh *et al*., 2003) [23]. In individuals with standard and low-normal blood pressure levels, a daily intake of 1000 mg of rebaudioside A could not result in clinically significant changes in blood pressure (Maki *et al*., 2008) [23].

3. Effects on obesity and metabolic syndrome: There has been a worldwide increase in the number of overweight or obese people, making it an issue of critical importance. Obesity is a metabolic disorder with various implications, including diabetes, cancer, cardiovascular disease, stroke, and sleep apnea; it is more than just being overweight. The risk of these diseases is much increased in obese people, and they can ultimately lead to a diminished quality of life. Abdominal obesity is a hallmark of metabolic syndrome, which increases one's chance of developing cardiovascular disease, according to Carrera-Lanestosa and co-workers, 2017 [8].

   Weight gain can be avoided by using non-nutritive sweeteners, according to studies (Ashwell, 2015) [4]. Consistent with this, *S. Rebaudiana*'s anti-obesity abilities have been demonstrated in scientific investigations. Consuming *Stevia* extract had no effect on how hungry people felt, according to a human study (Farhat *et al*., 2019) [15]. The reason for this was perhaps because of the substantial disparity in calorie consumption by those who didn't ingest Stevia extract, and those that did. This may also help to explain why, in a separate human experiment involving *Stevia* extract and sugar, those who took the stevia extract did not make up for their calorie deficit by eating more (Anton *et al*., 2010) [2].

4. Effects on the renal system: The kidneys are a vital organ performing the task of getting the body rid of waste end products and metabolic side or by-products. The kidneys have a role in controlling blood pressure, maintaining a healthy salt water equilibrium, and producing RBCs, according to Onopiuk and co-workers, (2015) [40]. Diabetic Kidney damage (DKD) is typically caused by diabetes (Reidy *et al*., 2014) [51]. The cells and tissues of the kidney experience alterations in response to high levels of sugar (Vallon and Komers, 2011) [67].

   Patients with ongoing kidney disorders who take stevia extracts have better premeal serum glucose, uric acid, and creatinine levels (Rizwan *et al*., 2018) [53]. The kidney function investigations (glomerular filtration rate or GFR and the renal effective plasma flow or ERPF) were not significantly altered by steviol. However, compared to controls, steviol infusion significantly increased urine output as a proportion of glomerular filtration rate and proportional salt and potassium output. Steviol may influence renal tubular salt and water transport, according to the evidence (Melis, 1997) [36].

5. Effects on chelation of transition metal ions and oxidative damage due to free radicals by neutralisation: Numerous medical conditions, such as malignancies, weight gain, diabetes, and neurological disorders, have been linked to the production of free radicals, which according to Garaschuk and co-workers, (2018) [18] and Vaiserman and associates, (2020) [63-64] are constantly generated in any living-organism as a consequence of metabolic activities or in response to an array of stresses. Naturally occurring (endogenous) defence against antioxidants is adequate under the physiological circumstances for neutralising free radicals and safeguarding against oxidative damages; however, as we age and consume an excessive amount of calories, we experience a disproportion between the generation and neutralisation of free radicals, which leads to persistent oxidative stress and the emergence of systemic inflammation Gaweł-Bęben and associates (2015) [20].

   A potential advantageous strategy for combating ageing and its concomitant pathogenic complications is the use of biologically active phytochemicals with antioxidant-rich substances and antibacterial anti-inflammatory properties according to Piskovatska and associates, (2019) [45] and Vaiserman and colleagues, (2020) [63-64]. Multiple research investigations suggested that *S. Rebaudiana*'s potential for healing and averting certain diseases may depend on its antioxidant qualities. Several phenolic compounds in *Stevia* leaves are capable of neutralising radicals that are harmful, reactive oxygen species and also chelating transition-metal ions, preventing the latter from participating in free radical formation via the Fenton reaction according to Ruiz-Ruiz and colleagues, (2015) [54] and Prata and co-workers, (2017) [47]. Stevia can be utilised to treat tissue pathological conditions associated with oxidative stress due to its anti-inflammatory and antioxidant characteristics (Ruiz Ruiz, 2014) [54].

   The extraction process, such as drying and extraction, determine whether or not *Stevia* extracts retain their potential as an antioxidant. In terms of their total extracted phenol concentration from *Stevia* leaves, glycol-aqueous extracts rank first, (with the maximum), followed by aqueous extracts and finally ethanol extracts according to Gawe-Bben and co-workers, (2015). The antioxidant potential of the leaves of *Stevia*, dried using several ways (high-temperature air drying at 180 degrees Celsius, heated air drying at 100 °C, drying under freezing temperatures, indirect sunlight drying) were compared, and it was shown that hot air drying at 180 °C was the best approach for maintaining high antioxidant potential. When compared to alternative drying processes, the antioxidant content was increased by a factor of two to three under this regime (Periche *et al*., 2015) [42].

6. Hidden metabolic organ: The microbes in the human gut are a sizable and intricate community of microbes. There exists proof to suggest that microbiota may be implicated in being overweight and the development of type 2 diabetes (Guinane and Cotter. 2013) [22]. The metabolic activity of gut microbes varies greatly between people and is impacted by a number of parameters, including the host genetic makeup of the initial microbial establishment or colonisation in the neonatal period, and exposure to medications and chemical compounds (Marchesi *et al*., 2016) [34]. However, research suggests that the most important element impacting the nature of the gut microbiota as a whole and the role it plays in the body is food patterns and dietary changes.
   Whilst steviol glycosides do not alter the composition of the intestinal microbiota, it is digested by microbes in the digestive tract. Because they possess enzymes that humans lack, the microbiota plays an indispensable part in the digestion of food (Lobach *et al*., 2018) [29]. Gardana and associates. (2003) [19] discovered that the capability to deglycosylate steviol glycosides appeared to be restricted to bacteria belonging to the Bacteroides genus. There was no evidence that any of the Clostridia, Bifid bacteria, coliform, Lactobacillus, or Enterococci cultures could process or breakdown stevioside or Reb A. Bacteroides is one of the most common types of bacteria in the human large intestine (Renwick and Tarka, 2008) [52], therefore individual differences in the rate at which it hydrolyzes steviol glycosides are likely to be small.
7. Effects on the proliferating tumor cells: Following cardiovascular disease, cancer (breast, intestines, ureters, and lungs) is the second most prominent reason of fatality globally. Identifying natural compounds that might aid prevent or treat cancer is thus of paramount importance (Ferlay *et al*., 2018) [16]. Steviol-mediated inhibition of different types of proliferating cancer cells was demonstrated by Chen *et al*., 2018 [10]. According to Boonkaewwan and associates, (2008) [7], when present in sufficiently significant amounts, stevioside has been demonstrated to be detrimental to the viability of the cells of colon cancer. Steviol is just as efficient as the anti-cancer medication 5-flourouracil (FU) at concentrations between 100 and 200 g/mL, and at 250 g/mL it is even more cytotoxic. This suggests that steviol could one day be used as a chemotherapeutic drug in the treatment of cancer (Chen *et al*., 2018) [10].
   It's additionally noteworthy to emphasise that, even at larger concentrations, Stevioside is not as harmful to normal cells. Stevioside was also found in another investigation to block replication of DNA and cause cancer cells to perish via the mitochondrial apoptosis. Protein synthesis of p21 and p53 is stimulated by steviol, but that of Cyclin D is inhibited. This improves the ratio of Bax to Bcl-2 in the cell. In reaction, Bax protein causes apoptosis marked by mitochondrial malfunction and the release of cytochrome c, which in turn activates caspases to degrade enzymes involved in DNA repair and genome maintenance, according to Paul and co-workers, (2012) [42]; Chen and associates, (2018) [10]. In breast cancer MCF-7 cell cultures, Stevioside exhibited potent anti-cancer activity (Paul *et al*., 2012) [42]. Takasaki and collaborators, 2009 [58] demonstrated that Isosteviol and other equivalent compounds inhibit the generation of an early Epstein Barr virus antigen that inhibits tumour growth. Therefore, studies like these confirm the value of steviol and other components of Stevia in treating cancer, as claimed by Mizushina *et al*., (2005) [37].
8. Anti-inflammatory or bactericidal actions of *Stevia rebaudiana* are extraordinary in that they might alleviate inflammation and kill bacteria. Jeong and collaborators, (2010) [25], Preethi and colleagues, (2011) [48], and Arya and associates, (2012) [3] found that *Stevia* extract inhibited the activities of numerous harmful microorganisms. Stevia's anti-bacterial characteristics was studied *in vitro* towards harmful microbes like *Bacillus subtilis, Pseudomonas fluorescence, Klebsiella pneumonia, Staphylococcus aureus, Proteus vulgaris,* and *Streptococcus pneumoniae,* with both the methanol-based extract from callus culture and the whole plant material showing substantial inhibition of infection. Antibacterial property was found in six leaf extracts and three flower extracts of *Stevia*, all of which were produced using different solvents according to Preethi and colleagues, (2011) [48] and Arya and associates, (2012) [3].
   According to a report by Gamboa and Chaves, (2012) [17] and by Singh and Dwivedi, (2014) [55], Stevia can kill germs within the mouth. Tooth decay, also known as dental caries, is a common problem that affects both young and old. Caries are linked to the presence of microorganisms and can be induced by a lack of care for one's teeth, a genetic susceptibility, a diet high in carbohydrates, or a deficiency of calcium and phosphorus (Contreras, 2013) [11]. After microbial colonisation, biofilm, and plaque deposition on teeth, poor oral hygiene and carbohydrate ingestion encourage active reproduction of mouth microbiota. *Streptococcus mutans, Streptococcus sobrinus,* and *Lactobacillus acidophilus* have all been demonstrated to be inhibited in their growth by S. *rebaudiana* in *in vitro* investigations.
   The build-up of plaque was evaluated in a human *in vivo* research following thrice-daily rinsing with a sucrose solution and once-daily rinsing with a *S. rebaudiana* solution. Plaque was reduced after rinsing with Stevia solution by 57.82% (as judged by the Silness-Loe index) and by 10.40% (as measured by the O'Leary plaque index) compared to sugar rinse (De Slavutzky, 2010) [12]. Based on the results of an investigation, by Giacaman and coworkers, (2013) [21], it appears that *Stevia* inhibits the growth of oral bacteria by reducing the number of viable microbial cells. It was suggested that Stevia be used to ward off dental cavities because of its antibacterial properties. Furthermore,

Vandana and co-workers (2017) [68] demonstrated that stevia effectively counteracted plaque and gingivitis.

9. Toxicity levels: Stevia dry extract at a daily dose of 4 mg/kg of body weight has been deemed safe for human consumption by the European Food Safety Authority's Scientific Committee on Food and the Food and Drug Administration (Lohner *et al*., 2017) [30].

   Asteraceae (Compositae) plant *Stevia rebaudiana* (Bertoni) yields steviol glycoside sweets. Chrysanthemum, chamomile, and sunflower can all cause hypersensitivity reactions. Based on their current taxonomic classification some popular media narratives and online platforms promulgated dietary precautions pertaining to stevia sensitivity. However, Stevia allergies are rare.

   A systematic study of literature was carried out to retrieve all evidence on allergic reactions to *Stevia* extracts or highly pure steviol glycosides to assess if stevia-based sweeteners ought to include allergy precautions.

   The rare incidents were reported prior to the 2008 launch of high-purity products, when numerous global regulatory bodies confirmed steviol glycosides' safety. Since 2008, no stevia-related allergies have been reported by stevia manufacturers or food allergy networks. Thus, warnings of sensitivity to highly pure stevia extracts are unsupported by the realm of science (Urban *et al*., 2015) [61].

   Additionally, none of the researchers (Nikiforov and collaborators, 2013, Uçar and colleagues, 2017) [38, 60] have found any negative side effects of Stevia affecting human health. It is important to remember, nonetheless, that not each and every Stevia commercial product in the market is of premium grade. In one investigation, the Raman spectroscopy analysis of six commercialised Stevia brands were analysed and three were found to contain minor levels of sodium saccharin and sodium cyclamate by Jentzsch and associates, (2016) [24]. The Raman spectroscopy analysis detects and identifies if the products are authentic as opposed to those that are counterfeit stevia, from amongst the commercialised brands.

Conclusions

Stevia rebaudiana is a perennial herb that is now grown world over as a source of a highly-potent zero-calorie sugar substitute. The sweet taste of Stevia comes from two principal steviol diterpene glycosides, (Stevioside and rebaudioside A). These are found in abundance within the leaves of the plant and are responsible for imparting a taste which is 150-450 times sweeter than sugar to human taste buds. Both processed sweet glycosides and crude Stevia preparations (containing 50% glycosides) find widespread application within the culinary industry. Several preclinical and clinical studies show that Stevia herbal preparations and their constituent components may have therapeutic and pharmacological uses because they are harmless and have health-promoting consequences. The several substances found in Stevia leaves, including glycosides, flavonoids, and fatty acids, contribute to the plant's wide range of beneficial effects. These components provide Stevia with its potent antibacterial, antioxidant, and immunomodulatory effects, as well as its ability to increase insulin secretion in diabetics and alleviate polycystic kidney disease. More study is required to determine which chemicals in Stevia are responsible for the observed effects and the molecular mechanisms by which they work. Stevia is a natural sweetener that has been shown to reduce food intake in addition to total cholesterol, triacylglycerols, and low-density lipoproteins, but the processes by which it does so remain to be determined.

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