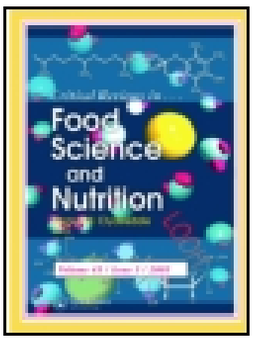

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Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health.

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Abstract

The leaves of *Stevia rebaudiana* Bertoni has nutrients and phytochemicals, which make it an adequate source for the extraction and production of functional food ingredients. Preclinical and clinical studies suggest therapeutic and pharmacological applications for stevia and their extracts because they are not toxic and exhibit several biological activities. This review presents the biological activity of *Stevia rebaudiana* Bertoni and their relationship to antidiabetic, anticariogenic, antioxidant, hypotensive, antihypertensive, antimicrobial, anti-inflamatory and anti-tumor activities. Consumption and adverse effects were also reviewed.

Key words: *Stevia rebaudiana*, extracts, steviol glycosides, bioactivity.

Introduction

Stevia rebaudiana is native to the northeast of Paraguay. Actually is cultivated in other regions of the world, including North America, Asia and Europe (Lemus-Mondaca et al., 2012). Stevia consists of a group of annual and perennial herbs, subshrubs, and shrubs that occur in mountain regions, open forests, borders of rivers, and dry valleys. Its first botanical description is attributed to M. S. Bertoni. The plant was first called *Eupatorium rebaudianum* Bert. in honor of Rebaudi, the first chemist to study the chemical characteristics of the substances extracted. Its name was later changed to the current one, and it was recommended not only for food production, but also for the medicinal effects that were attributed to it. The genus Stevia included 230 species but only *S. rebaudiana* gives the sweet essence. Botanically *S. rebaudiana* is described as a perennial low shrub with extensive roots, brittle stems, and small, elliptical leaves. Under some environmental conditions and management situations, it behaves as an annual or a mixture of plants of both types. The cultivated plants are reported to be more vigorous. Some other related species include *S. eupatoria*, *S. lemmonii*, *S. micrantha*, *S. ovata* var. *texana*, *S. plummerae*, *S. plummerae* var. alba, *S. rhombifolia*, *S. salicifolia*, *S. serrata*, *S. viscida*, *S. commixta*, *S. satureiaefilia, S. leptophylla, S. myriadenia, S. ophryphylla, S.selloi, S. nepetifolia, S. oligophylla, S. origanoides, and S. triflora* (Kumar et al., 2011).

Several natural products have been isolated from *S. rebaudiana*, the best known of which are the steviol and its glycosides, stevioside, rebaudioside (A to F), steviolbioside, dihydroisosteviol, rubusoside, and dulcoside A (Savita et al. 2004). Nutrients in Stevia include water (80-85%), protein, fiber, monosaccharaides, lipids, essential oils, vitamin C, β-carotene, vitamin B2, vitamin B1. Include antioxidants compounds like apigenin, quercetin, isoquercitrin, luteolin,

miocene, kaempferol, chlorogenic acid, and caffeic acid. Also, include minerals as cobalt, magnesium, iron, potassium, and phosphorus (Gupta et al 2013].

Stevia has been used for various purposes throughout the world; the Guarani tribes used *S. rebaudiana*, as a sweetener in herbal infusions. Japan was the first country in Asia to market steviol glicosides in the food and drug industry. Since then, cultivation has expanded to China, Malaysia, Singapore, South Korea, Taiwan, and Thailand (Lemus-Mondaca et al., 2012).

Steviol glycosides have been used to replace sucrose, for treatment of diabetes mellitus, obesity, hypertension, and caries (Jaroslav et al., 2007). Studies have suggested that stevyol glycosides exhibit therapeutic benefits with antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, antidiarrheal, diuretic, and inmunomodulatory effects (Chatsudthipong and Muanprasat, 2009). The leaves of stevia have functional and sensory properties superior to those of many other sweeteners (Jaroslav et al., 2007).

The purpose of this review is to bring together a selection of basic data coming from scientific studies on stevia. With emphasis on functional and health-promoting properties.

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Antidiabetic activity

Diabetes mellitus is characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion or insulin action (Barcelo and Rajpathak, 2001). Without enough insulin, the cells cannot absorb sufficient glucose from the blood; hence, blood-glucose levels increase (hyperglycemia). If glucose level in blood remains high during a long period, this can result in damage to kidneys, liver, nerves, and cardiovascular system (Pari and Saravanan, 2004). The two types of diabetes are insulin-

dependent diabetes mellitus (IDDM), or type I, and non-insulin-dependent diabetes mellitus (NIDDM), or type II. The WHO classification also recognized malnutrition-related diabetes mellitus and gestational diabetes. According to Suanarunsawata et al. (2004) several ethnobotanical studies report that more than 500 plants are used for diabetes mellitus treatment, and more than 1200 species exhibit hypoglycemic activity (Okyar et al., 2011). Before the discovery of insulin by Banting and Best in 1922, the only options for diabetes treatment were those based on traditional practices (Ribnicky et al., 2006). Plants have been used as dietary adjuvant and for treatment of diseases even without knowledge on their functions and constituents. Although numerous synthetic drugs were developed for the treatment of diabetes mellitus but the safe and effective treatment paradigm is yet to be achieved (Patel et al., 2012). Among those plants used for the treatment of diabetes is *S. rebaudiana* (Li et al., 2004).

Suanarunsawata et al. (2004) evaluated the glycemic effect of stevioside (250.0 mg/kg body weight) and *S. rebaudiana* extract (4660.0 mg/kg body weight) in diabetic rats induced with streptozotocin for eight weeks. These authors observed that plasma glucagon (PG) level was slightly but significantly raised in normal rats fed with stevioside since the third week. *S. rebaudiana* extract had no effect on the PG in normal rats. Stevioside had no effect on the PG in rats, whereas *S. rebaudiana* significantly reduced the PG in diabetic rats from the second week until the end of experiment. The glycemic effect of stevioside and *S. rebaudiana* extract was correlated with the alteration of serum insulin levels. There were no changes in normal rats fed with either stevioside or *S. rebaudiana*. In contrary, stevioside and *S. rebaudiana* improved the serum insulin in diabetic rats. Suanarunsawata et al. (2004) concluded that stevioside potentiated insulin release in diabetic induced rats, a reduction of insulin-induced glucose uptake was

apparent as well; thereby no anti-hyperglycemic effect was apparent. *S. rebaudiana* decreased PG in diabetic rats by the mechanism of an improvement of insulin and suppression of glucagon level. Stevioside containing in *S. rebaudiana* may indirectly contribute to anti-hyperglycemic action of *S. rebaudiana* in diabetic rats via its effect to potentiate insulin release.

In another study, Dutta et al. (2010) compared the hypoglycemic effect of Stevia and metformin hydrochloride in a short-term study. The authors concluded that the continuous treatment with Stevia and metformin produced a significant ($p<0.001$) reduction of the blood-glucose level and in diabetic rats induced with streptozotocin. Among the doses of aqueous extract (10%) of Stevia (2.0, 5.0, and 10.0 mL/kg of body weight), 10.0 mL/kg of body weight was most potent. Klepser and Kelly (1997) established that metformin hydrochloride reduces the blood-glucose levels by stimulating glucose uptake of skeletal muscle. According to the results obtained by Dutta et al. (2010) and Suanarunsawata et al. (2004), the extract of *S. rebaudiana* could exhibit the same mechanism of action as metformin.

Curi et al. (1986) evaluated the effect of aqueous extracts of *Stevia rebaudiana* leaves on a glucose tolerance in 16 healthy volunteers. Aqueous extracts of 5.0 g of leaves were administered to volunteers at regular 6-h intervals for 3 days. Glucose tolerance tests were performed before and after extract administration. A second group of six healthy volunteers who ingested an aqueous arabinose solution was also studied. The extract of *Stevia rebaudiana* increased glucose tolerance. The extract significantly decreased plasma glucose levels during the test and after overnight fasting in all volunteers from a basal value of 82 mg/dL at the beginning of the analysis to 68 mg/dL after 3 h of ingestion. Acording to Curi et al (1986) it is possible that Stevia extract, by increasing the mitochondrial respiration rate and inhibiting the

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gluconeogenesis pathway, can indeed lead to hypoglycemia. Nevertheless, a possible effect of this plant of insulin secretion or peripheral action should be considered.

Jeppesen et al. (2000) elucidated the impact of stevioside and its aglucon steviol on insulin release from normal mouse islets and the beta-cell line INS-1. Both stevioside and steviol (1 nmol/L to 1 mmol/L) dose-dependently enhanced insulin secretion from incubated mouse islets in the presence of 16.7 mmol/L glucose ($P < .05$). The insulinotropic effects of stevioside and steviol were critically dependent on the prevailing glucose concentration, ie, stevioside (1 mmol/L) and steviol (1 micromol/L) only potentiated insulin secretion at or above 8.3 mmol/L glucose ($P < .05$). According to these authors, the insulinotropic effects of both stevioside and steviol were preserved in the absence of extracellular Ca^{2+}. During perifusion of islets, stevioside (1 mmol/L) and steviol (1 micromol/L) had a long-lasting and apparently reversible insulinotropic effect in the presence of 16.7 mmol/L glucose ($P < .05$). The authors also determined if stevioside and steviol act directly on beta cells and on INS-1 cells. Stevioside and steviol both potentiated insulin secretion from INS-1 cells ($P < .05$). Neither stevioside (1 to 100 micromol/L) nor steviol (10 nmol/L to 10 micromol/L) influenced the plasma membrane K^+ adenosine triphosphate ((K^+)ATP)-sensitive channel activity, nor did they alter cyclic adenosine monophosphate (cAMP) levels in islets. Jeppesen et al. [18] concluded that stevioside and steviol stimulate insulin secretion via a direct action on beta cells. The results indicate that the compounds may have a potential role as antihyperglycemic agents in the treatment of type 2 diabetes mellitus.

More recently Mishra (2011) performed an analysis of anti-diabetic activity of *S. rebaudiana* extract. Fifteen patients were introduced; nine patients were woman, six patients were man, and

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the age group was 25-60 years. During the experimental period, the patients did not use hypoglycemic drugs. Stevia leaf powder (0.5-1.0 g) was used in place of sugar in tea/coffee and milk intake. Feeding trial was for 45 days. First 15 days patient received hypoglycemic drug and their postprandial glucose level (PGL) and fasting glucose level (FGL) were measured then for next 15 days they were not given any medicine under normal diet and their PGL and FGL were measured and then for further 15 days they were given Stevia leaf three times (3.0 g each time) a day with tea tea/coffee or milk intake and their glucose levels were measured. During the first 15 days the FGL was 153.54 mg/dL and PGL was 189.56 mg/dL (means). For next 15 days, patients were asked to stop taking the hypoglycemic drug under normal diet and, under supervision of a physician, their FGL and PGL were checked, which were 208.6 mg/dL and 283 mg/dL (means), respectively. There were significant differences in both FGL and PGL that showed that while taking the hypoglycemic drug, their glucose levels were under control. For 15 more days, patients were given *Stevia* leaf powder three times a day, and FGL and PGL levels were checked, which were 195.7 mg/mL and 271.3 mg/mL (means), respectively. The authors concluded that *S. rebaudiana* extract decreased glucose levels but not statistically significant and for more significant result large sample size should be taken in consideration under controlled condition and for extended period of time.

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Anticariogenic activity

Dental caries are considered to be a localized disease that results from the metabolic processes of the biomass in contact with the tooth surface, and the diet provides nutritional requirements and therefore, the energy to the microorganisms of the oral microbiota (Vitery et al., 2010). Stevia, as

an added sweetener, has been investigated recently and was shown to be non-cariogenic. Since the first signs of its anticariogenic properties, several studies have been carried out. These studies confirmed this property and describe the mechanisms of how this happens (Contreras, 2013). According to Contreras (2013), it is possible to group these mechanisms into three groups.

1) Antibacterial effect on microorganisms associated with the production of tooth decay

The complexity of the bacterial community found on the surfaces of the teeth makes it difficult to associate specific groups of bacteria with the cause of teeth decay. However, *Streptococcus mutans* and *Lactobacillus acidophillus* are found in almost all tooth decay lesions, and their proportion in the plaque and saliva is positively related to the frequency and activity of tooth decay (Thylstrup and Fejersko, 2000). It is also known that *Streptococcus sobrinus* is involved in the development of tooth decay (Okada et al., 2005). Mohammadi-Sichani et al. (2012) studied the *in vitro* effect of stevia extracts in different solvents on *Streptococcus mutans* using tetracycline (1%) as a positive control. The extracts of stevia in acetone, ethanol, and methanol had a dose-dependent antibacterial activity, with the first two presenting the largest inhibition zones against that bacterium, reaching values of 28.7 mm (acetone) and 27.0 mm (ethanol), both in a 100 mg/ml concentration, compared to the positive control, which had an inhibition zone of 10 mm. Vitery et al. (2010) compared the effect of different concentrations of stevia extracts in water, methanol, ethanol, hexane, and ethyl acetate on strains of *Streptococcus mutans* and *Lactobacillus acidophilus* using vancomycin as a positive control. The stevia extract that showed the best results in the inhibition of growth, both for *Streptococcus mutans* and *Lactobacillus acidophilus*, was the hexanoic acid, in which after 48 hours, inhibition halos were formed with an average of 14.5 mm with *Streptococcus mutans* and 15.5 mm with *Lactobacillus acidophilus*

at a concentration of 50 mg/ml. The other solvents also showed activity against the studied bacteria, although higher concentrations were necessary. Gamboa et al. (2012) evaluated the antibacterial effect of extracts from leaves of stevia in hexane, methanol, ethanol, ethyl acetate, and chloroform on 12 strains of streptococcus (including *Streptococcus mutans*) and four strains of lactobacillus (including *Lactobacillus acidophilus*), using vancomycin (180 μg/ml) and azithromycin (150 μg/ml) as positive controls, which managed inhibition zones with values between 18 mm and 25 mm. The zones of inhibition produced by the five extracts at the minimum inhibitory concentration (MIC) for the 16 strains were variable, with values ranging from 9 mm to 17.3 mm. The best performance was found for the hexanoic extract, whose MIC was 30 mg/ml, which had similar values to those achieved with ethanol and methanol (MIC = 120 mg/ml). In experiments of tooth decay in rats, Das et al. (1992) found significant differences in the count of sulcal caries and *Streptococcus sobrinus* between the group of sucrose and the group of stevia sweeteners. There were no significant differences between stevioside and rebaudioside A. The study came to the conclusion that neither of them is cariogenic. Noting the *in vitro* effect of the extracts of stevia to 20% on *Streptococcus sobrinus,* Triratana et al. (2006) registered an inhibition in the growth rate (50% inhibition) and a decrease in the production rate of the acid by the bacteria. It was concluded that stevia extract had an inhibitory effect on the caries-producing properties of *Streptococcus sobrinus*.

2) Low acidogenic potential

Oral bacteria, which are found in the dental plaque, easily metabolize sucrose, and the result is the release of acids. These acids are responsible for the demineralization of dental tissues in the dynamic process of caries. Goodson et al. (2010) evaluated the cariogenic potential of

rebaudioside A in an *in vivo* study, and compared the effect on the pH of a solution of rebaudioside A and sucrose (both 4.7%). The rinse with rebaudioside A showed a minimum pH of 6.92, which was significantly higher than the sucrose (pH 5.62), verifying a low acidogenic potential and complying with the criteria established by the Food and Drug Administration (FDA) for a non-cariogenic sweetener. Giacaman et al. (2013) also noted that saccharin, stevia, and sucralose induced a significantly lower acidogenicity throughout the entire experiment. Regarding enamel demineralization, all the tested sweeteners, including stevia, showed a statistically significantly lower percentage of loss of surface hardness compared to the positive control, sucrose.

3) Antiplaque effect

Most of the diseases in the mouth begin with the formation of dental plaque, which are complex structures that are associated with similar microorganisms and different bacterial species. In an *in vivo* experiment, De Slavutzky (2010) measured the accumulation of dental plaque after mouthwashes with a solution of sucrose and one of stevia for 5 days. The accumulation of plaque after the mouthwashes with stevia was 58% less than with the sucrose mouthwashes when measured with the Silnessï Loe index and 10% less when it was measured with the O'Leary index. In two *in vitro* experiments that aimed to determine the inhibitory effect of stevia extracts (10% and 20%) against *Streptococcus sobrinus*, a decrease in the hydrophobicity of the surface, extracellular polysaccharides production, and the adhesion of bacteria to the plaques coated with saliva was noted (Suwannawong et al., 2004; Triratana et al., 2006).

According to Contreras (2013), possible explanations for these properties are based on its contents. This plant is rich in flavonoids and terpenes, the phytochemicals present in stevia are

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austroinullin, ß-carotene, dulcoside, niacin, rebaudi oxides, riboflavin, steviol, stevioside, and thiamine (Mandal and Mada, 2013). These nutritious substances affect the microbial flora of the mouth. Furthermore, the content of tannin, xanthines (theobromine and caffeine), and flavonoids have antiplaque activity (Söderling et al., 2008). In addition, the leaf extracts of stevia and its major polyphenolic constituents, stevioside and rebaudioside A, are not cariogenic. Stevioside has a slight effect on the enzymes responsible for the decomposition of sugars, a discreet inactivation of the dextransucrase and a light static-effect on the cariogenic bacteria (Yabu et al., 1997).

The application of Stevia in the dental treatment is a barely explored field. In order to materialize their contribution to this area, further studies are needed on the isolation, characterization and identification of substances present in the extracts. It has to be found the solvent that achieve the best use of the active components of this plant and make it biocompatible; the concentration has to be selected to suits the standards of acceptable daily intake and make it effective at the same time, and has to be found a mean of administration considering the time spent at the site of action so that the active compound will achieve the desired effect.

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Antioxidant activity

Recently, much attention has been focused on dietary natural antioxidants capable of inhibiting oxidative stress mediated by reactive oxygen radicals, which is involved in several pathological diseases, such as cancer, atherosclerosis, diabetes, inflammation, and aging. Exogenous dietary antioxidants, called nutraceuticals, are capable of scavenging free radicals, and have shown promise in preventing certain diseases. Dietary antioxidants can increase cellular defenses and

can help prevent oxidation damage to cellular components (Ghanta et al., 2007). Different authors have reported the antioxidant activity of stevia. Jahan et al. (2010) performed four complementary test systems: DPPH free-radical scavenging, reducing power, total phenolics, and total flavonoids concentration in order to study the antioxidant capacities of different organic and aqueous soluble materials of *S. rebaudiana* leaves. The IC_{50} (concentration of sample where the response is reduced by half) values of the 80% ethanol extract and water-soluble fractions were found to be 8.02 and 43.81 ε g/mL, respectively. In the same test, the standard compound (ascorbic acid) exhibited an IC_{50} value 4.21 ε g/mL. In reducing power tests, the maximum absorbance for the 80% ethanol extract and water-soluble fractions was found to be 1.071 nm and 1.555 nm, respectively, compared to the absorbance of ascorbic acid as a standard (1.374 nm), this indicates that the ethanol extract has a higher reducing power than ascorbic acid. Total phenolic concentrations in the 80% ethanol extract and water-soluble fractions were 65.21 and 41.49 mg of gallic acid equivalent/g of dry extract, respectively. Total flavonoid concentrations in the 80% ethanol extract and water-soluble fractions were 125.64 and 101.45 mg of quercetin equivalent/g of dry extract, respectively. The results obtained by these authors revealed that the 80%-ethanol extract exhibited the most significant antioxidant activity.

In another study, Shivanna et al. (2012) identified by mass spectrometry the major phenolic compounds in stevia powder extracted with ethanol: dicaffeoylquinic acid, chlorogenic acid, quercetin 3-O-xyloside, apigenin-7-O-glucoside, 3,4-dimethoxycinnamic acid, luteolin-7-O-rutinoside, and caffeic acid. Previous studies noted that the importance of hypoglycemic components of stevia is due to the rebaudioside A and stevioside concentrations in leaves [38]. However, Shivanna et al. (2012) did not identify these compounds in the polyphenol extract they

studied, which explains the involvement of polyphenolic compounds in preventing diabetic and its complications caused by the streptozotocin model (Bennett and Pegg, 2004; Imaeda et al., 2002; Wheeler et al., 2008). The same authors compared the antioxidant properties of stevia leaves with other commercial antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butyl hydroquinone. Stevia leaves exhibited better antioxidant properties, such as free-radical scavenging (EC_{50} = 2.6 μg) and inhibition of lipid peroxidation (EC_{50} = 10.6 g).

Ghanta et al. (2007) studied the oxidative damage-prevention activity and antioxidant potential of *S. rebaudiana*. At 0.1 mg/mL, the ethyl acetate extract (EAE) of the crude 85% methanolic extract (CAE) of *S. rebaudiana* leaves inhibited DNA strand scission by ÅOH, generated via a Fenton reaction, on pBluescript II SK (ï) DNA. Its efficacy was better than that of quercetin. The radical-scavenging capacity of CAE was evaluated by the DPPH test (IC_{50} = 47.66 ± 1.04 ε g/mL). EAE derived from CAE scavenged DPPH (IC_{50} = 9.26 ± 0.04 ε g/mL), $ABTS^{+}$ (IC_{50} = 3.04 ± 0.22 ε g/mL) and ÅOH (IC_{50} = 3.08 ± 0.19 ε g/mL). Additionally, CAE inhibited lipid peroxidation induced with 25 mM $FeSO_4$ on rat liver homogenate as a lipid source (IC_{50} = 2.1 ± 1.07 mg/mL). The total polyphenols and total flavonoids of EAE were 0.86 mg gallic acid equivalent/mg and 0.83 mg of quercetin equivalent/mg, respectively. Flavonoids, isolated from EAE, were characterized as quercetin-3-O-arabinoside, quercitrin, apigenin, apigenin-4-O-glucoside, luteolin, and kaempferol-3-O-rhamnoside by LC-MS and NMR analysis. Ghanta et al. (2007) concluded that *S. rebaudiana* may be useful as a potential source of natural antioxidants.

Methanolic and ethanolic extracts were used to estimate antioxidant activity of *S. rebaudiana* leaves (Sutradhar et al., 2013). The antioxidant activity was studied using the reducing power of

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S. rebaudiana extracts by the Oyaizu method, the content of total phenols was estimated by a standard method using the Folinï Ciocalteu reagent, and total flavonoids was determined by the aluminum chloride colorimetric method. Absorbance of different concentrations of methanolic extract, ethanolic extract, and standard butylated hydroxyl toluene solutions was measured at 700 nm and was compared. The extracts showed similar reducing power capacity at different concentrations ranging from 100 to 1000 μg /ml. The total phenolic content of methanolic and ethanolic extracts of *S. rebaudiana* leaves was found to be 280 mg TAE/g and 810 mg TAE/g, respectively. The phenolic compounds are known to have direct antioxidant properties. The total flavonoids content of methanolic and ethanolic extracts was found to be 12 mg CE/g and 15 mg CE/g, respectively. The tannins and flavonoids present in the leaves extract may be responsible for antioxidant activity.

Jahan et al. (2010) studied the antioxidant activity of *S. rebaudiana* Bert. leaves from Bangladesh (Table 1). IC_{50} values for DPPH free radical scavenging activity ranged from 8.02 μg /mL (ethanol 80%) to 44.61 μg /mL extract (hot water). In the reducing power test, the maximum absorbance values ranged from 1.5552 (water) to 0.5684 (dichloromethane). In both studies ascorbic acid was used as standard. In DPPH free radical scavenging tests a high absorption indicates a stronger antioxidant capacity of the samples.

Total phenolic concentrations in 80% ethanol extract (at room temp.) and its different fractions ranged from 25.36 ± 0.34 to 65.21 ± 0.97 and those in different hot extracts were in the range 15.33 ± 0.78 to 36.95 ± 0.09 mg/g gallic acid equivalent, respectively. The total flavonoids concentration of 80% ethanol extract and its fractions ranged from 34.26 ± 0.79 to 125.64 ± 1.07,

and the range for hot extracts was 23.56 ± 0.89 to 76.94 ± 0.35 mg/g quercetin equivalent/g of dry extract, respectively. The results revealed that 80% ethanol extract exhibited the highestantioxidant activity, followed by its water and 1-butanol extracts, and hot-methanol and hot-water extracts. Jahan et al. (2010) concluded that *S. rebaudiana* leaves from Bangladesh have significant potential as a natural antioxidant.

Tavarini and Angelini (2013) studied the effect of harvest time, experimental site, and crop age on the steviol glycosides and on the antioxidant properties of stevia leaf extracts. The experiment was conducted over two growing seasons at two sites in the northeastern plain of Italy. The results showed that all analyzed factors played an important role in defining the steviol glycosides profile and the antioxidant properties of stevia extracts. A high level of phenols (78.24 mg GAE/g DW by the Folin-Ciocalteu method) and high antioxidant activity (812.6 μmol Fe^{2+}/g DW by FRAP assay) were observed. The inhibition of DPPH free radicals was evaluated and an IC_{50} mean value of 250 μg/L was obtained. Significant relationships between the total antioxidant capacity and the analyzed compounds were found. The authors concluded that it was possible to obtain very high steviol glycosides yields thanks to the long days during the spring/summer season, and that the harvest time played a key role in determining the stevia quality, influencing the rebaudioside A/stevioside ratio.

The relationship between the antioxidant activities of *S. rebaudiana* and its abrogating effect on insulin resistance was evaluated by Shivanna et al. (2012). These authors evaluated the oxidative stress in livers of streptozotocin-induced diabetic rats (60 mg/kg body weight) by measuring the thiobarbituric acid reaction substances (TBARS), conjugated dienes, and hydroperoxides, which are products of lipid peroxidation. Peroxidation was reduced significantly in rats previously fed

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with stevia leaf powder and extracted polyphenols (4.0 g of leaf powder or phenolic extract in 96 g of dry diet) by 25% and 30% in the liver compared to the diabetic group. Since high blood glucose results in oxidation, hyperglycemia causes high reactive oxygen species (ROS) production and, in turn, leads to high malondialdehyde levels in tissues (Das et al., 2000). Increments of lipid peroxidation have been found to be involved in tissue damage in diabetes (Ahmed et al., 2006; Can et al., 2004; Mahboob et al., 2005.]. Shivanna et al. (2012) observed that streptozotocin administration resulted in a significant decrease in vitamin-C content (27%), an increase in vitamin-E levels (130%), and an increase in glutathione or the glutathione/glutathione disulfide ratio (45%) compared to rats pre-fed with stevia leaf powder and extracted polyphenols, which did not alter the levels of these antioxidants in plasma. Streptozotocin administration also resulted in a significant reduction in the hepatic antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) by 50%. When rats were previously fed with stevia leaf powder and extracted polyphenols, the enzyme activity was restored to normal. According to these authors, pre-treatment with stevia stimulated SOD and CAT to reverse oxidative damage. The results indicate that stevia showed significant protection against the oxidative damage in livers of streptozotocin-induced diabetic rats.

Singh et al. (2013) evaluated the antihyperglycemic and antioxidative properties of methanolic extracts of *S. rebaudiana* leaves in alloxan-induced diabetic mice. Alloxan is a specific toxin that causes massive destruction of the pancreatic β-cells, provoking a state of primary deficiency of insulin without affecting other islet types, thus creating a hyperglycemic condition. However, after continuous treatment of diabetic mice with *S. rebaudiana* for 21 days, the authors observed a considerable reduction of 39.84% (193.0 down to 116 mg/dL) in the sugar level. In the liver,

kidney, and pancreas, the authors estimated changes in SOD, glutathione peroxidase (GSH-Px), reduced glutathione (GSH), and TBARS. According to Singh et al. (2013), treatment with leaf extract of *S. rebaudiana* did not show an improvement in the SOD level of pancreatic and renal tissues. However, hepatic level was normalized in the case of SOD. Moreover, treatment with the leaf extract caused a significant increase in the level of GPx, suggesting its compensatory role in reducing the production of hydrogen peroxide, thus diminishing the toxic effects of free radicals produced by it in various secondary reactions. After the extract treatment for 21 days, a significant increase in the GSH level was observed, which indicates that the leaf extract of *S. rebaudiana* has the potential to increase the biosynthesis of GSH, thereby reducing oxidative stress. According to Sabu and Kuttan (2002), the diabetogenic effect of alloxan is due to excess production of ROS, leading to cytotoxicity in pancreatic ɓ-cells, which reduces the synthesis and the release of insulin while affecting organs, such as the liver, kidney, pancreas, and hematopoietic system. In their study, Singh et al. (2013), using alloxan induction, reported an increase in the level of TBARS concentrations in diabetic mice. However, treatment with methanolic leaf extract significantly reduced the level of TBARS. The multiple effects observed by Singh et al. (2013) could be due to the presence of some biomolecules present in the plant extract that may have stimulated the β-cells of Langerhans to release insulin, leading to an improvement in the carbohydrate-metabolizing enzymes, and thus establishing normal blood-glucose levels (Hossain et al., 2011). According to diverse studies, extracts of leaves of *S. rebaudiana* exhibit the presence of various phytochemicals, such as alkaloids, flavonoids, phenols, steroids, and tannins (Preethi et al., 2011; Söderling et al., 2008). Most of these compounds could have a marked antioxidant effect on cells, tissues, or organs.

Hypotensive and antihypertensive activity

The frequency of lifestyle-related diseases is steadily increasing, particularly of hypertension, a risk factor for cardiovascular diseases, such as coronary heart disease, peripheral arterial disease, and stroke. Indeed, cardiovascular diseases are the primary cause of morbidity and mortality in Western countries, with hypertension affecting about 20% of the worldôs adult population (Miguel et al., 2011). Essential hypertension is defined as an increase in blood pressure above certain measured levels. The definition of high blood pressure begins at a systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg. High blood pressure results in pathological changes that accrue in medium-sized and small arteries that cause further increases in blood pressure. The pathology is a thickening of the walls of these blood vessels so that the diameter of the vessels is effectively diminished, which causes the heart to work harder to pump enough blood to meet the demands of all the tissues, thus increasing the risk of a heart attack or stroke (Gupta et al., 2013).

Stevia has previously been shown to reduce blood pressure in studies in animals. According to Lee et al. (2001), intraperitoneal injection of stevioside (25 mg/kg) had an antihypertensive effect. In isolated aortic rings from normal rats, stevioside dose-dependently relaxed the vasopressin-induced vasoconstriction in both the presence and absence of the endothelium. However, stevioside had no effect on phenylephrine- and KCl-induced phasic vasoconstriction. In addition, stevioside lost its influence on vasopressin-induced vasoconstriction in Ca^{+2}-free medium. Stevioside caused vasorelaxation via an inhibition of Ca^{+2} influx into the blood vessel, which was confirmed in cultured aortic smooth muscle cells. Using 10^{-5} M methylene blue for 15

minutes, stevioside could still relax 10^{-8} M vasopressin-induced vasoconstriction in isolated rat aortic rings, showing that this vasorelaxation effect was not related to nitric oxide. Chan et al. (1998) studied the effect of intravenously introduced stevioside on the blood pressure of spontaneously hypertensive rats (SHR). In the conscious SHR, the hypotensive effect on both systolic and diastolic blood pressure was dose-dependent for intravenous doses of 50, 100, and 200 mg/kg. Serum dopamine, norepinephrine, and epinephrine levels did not change significantly 60 minutes after intravenous injection of stevioside at 100 mg/kg in anesthetized SHR. Stevioside, given intravenously to conscious SHR, was effective in blood pressure reduction and there was no change in serum catecholamines in anaesthetized animals with this natural compound.

Some studies have shown the clinical efficacy of stevia leaves in reducing chronic hypertension by relaxing arteries and helping to prevent the build-up of calcium on artery walls. Vasorelaxation induced by stevioside was studied by Chan et al. (1998) in a year-long randomized, double-blind, placebo-controlled study of 106 hypertensive subjects who consumed capsules containing either stevioside (750 mg daily) or placebo. Beginning after 3 months and persisting throughout the remaining 9 months of the study, the subjects consuming stevioside exhibited significantly greater decreases in systolic and diastolic blood pressures. No significant adverse effects occurred. Hsieh et al. (2003) reported, in a longer 2-year study, that 1500 mg stevioside daily produced significantly greater decreases in systolic and diastolic blood pressures in subjects with mild hypertension compared to placebo. Sharma et al. (2009) studied the effect of consumption of stevia extract on 20 selected hypercholesterolemic women (40-60 years), and found that consumption of 20 mL extract (165 mg/mL) in a glass of water (200 ml) helped in the

reduction of cholesterol, triglyceride and LDL-C significantly while an increased in HDL-C was noted which is desirable. Thus, it is concluded that stevia extract have hypolipidemic effect and can be used to reduce the risk of CVD.

Antimicrobial activity

S. rebaudiana Bertoni has the ability to inhibit the growth of certain bacteria, which helps to explain its traditional use in treating wounds, sores, and gum disease. It may also explain why the herb is advocated for anyone who is susceptible to yeast infections or reoccurring streptococcal infections, two conditions that seem to be aggravated by white sugar consumption. The biological activity of Stevia compounds has been studied by Tomita et al. (1997). They have studied bactericidal activity of a fermented hot-water extract from Stevia toward enterohemorrhagic *Escherichia coli* and other food-borne pathogenic bacteria like *Salmonella typhimurium*, *Bacillus subtilis*, and *Staphylococcus aureus*. The medicinal properties are attributed to the primary and secondary metabolites synthesized by the plants (Sharma et al., 2009). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethanolic extracts from plants in India (Tomita et al., 1997). The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Faizi et al., 2003).

Preethi et al. (2011) performed a preliminary phytochemical analysis of crude extracts of leaves and flowers of *S. rebaudiana* and observed the presence of various phytochemicals such as alkaloids, flavonoids, phenols, steroids, and tannins. Alkaloids were present in high

concentration in chloroform and hexane leaf extracts, and a moderate amount of flavonoids were present in methanol, chloroform, and petroleum ether flower extracts and in ethanol leaf extracts. Flavonoids were present in high concentrations in ethanol leaf extracts. Higher concentrations of phenols were also present in the ethanol leaf extracts when compared to the other extracts. Lower amounts of phenols are present in the flower extracts. Higher concentrations of steroids were present in ethanol and hexane leaf extracts and in chloroform flower extracts. Lower amounts of steroids are present in the methanol and petroleum ether extracts of flowers. Ethanol leaf extracts contain high concentrations of tannins when compared with the other extracts. Methanol and petroleum ether flower extracts contain fewer amounts of tannins. The preliminary phytochemical studies were of pronounced importance because the crude drugs possess varied compositions of secondary metabolites. The same authors evaluated the antibacterial activity of the extracts against several microorganisms, including *Pseudomonas fluorescens*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Streptococcus pneumonia*. All nine crude extracts showed good antibacterial activity. Some of the extracts, like the petroleum ether leaf and flower extracts gave very low minimum inhibitory concentration (MIC) values. Petroleum ether flower extract gave the lowest MIC values (0.390-1.562 ε g/mL) against all the bacterial isolates tested. The lowest MIC value (0.390 ε g/mL) was recorded against *B. subtilis*, *Pseudomonas fluorescens*, and *S. pneumoniae*. Methanol flower extracts showed MIC values in the range of 0.781-6.25 ε g/mL, whereas leaf methanol extract showed 3.125 and 12.5 ε g/mL values, respectively, for all organisms. Of all the extracts tested, the lowest MIC values were recorded in all organisms for the flower rather than leaf extracts.

Khan et al. (2012) used solvents such as water, ethanol, methanol, hexane, acetone, chloroform,

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and ethyl acetate to extract the secondary metabolites from leaves, stems, and roots of *S. rebaudiana* and evaluated their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using tetracycline as a control. All the extracts were effective against the pathogens used. The most effective extracts from leaves were methanol, ethanol, chloroform, and ethyl acetate. Among the extracts of stems, the most effective were methanol, chloroform, acetone, and ethyl acetate. Among the extracts of roots, the most effective were methanol, chloroform, and acetone. According to these authors, the extracts obtained from leaves showed greater antimicrobial activity than stem and root extracts. These results demonstrate that antimicrobial activity is correlated with the content of secondary metabolites present in different parts of the plant, which varies depending on the part of the plant.

Gosh et al. (2008) evaluated the antimicrobial activity of *S. rebaudiana* leaf extracts (i.e., petroleum ether, cyclohexane, chloroform, water, acetone, and ethanol) against 10 selected pathogenic as well as food-spoilage fungal (*Alternaria solani*, *Helminthosporium solani*, *Aspergillus niger*, and *Penicillium chrysogenum*) and pathogenic bacterial (*Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) isolates, using streptomycin and cotrimazole as controls. These authors found that petroleum ether extracts at 250 μg/mL (MIC) inhibit the growth of *E. coli* and *S. aureus* (by the plate dilution method) among bacteria and *P. chrysogenum* among fungi. Among all extracts, petroleum ether exhibited the best antimicrobial potential followed by water, chloroform, cyclohexane, acetone, and ethanol. This shows that extracts of *S. rebaudiana* act on a wide spectrum of microorganisms.

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While most of the extracts obtained with organic solvents showed antimicrobial activity, only those that are obtained with water or ethanol are used in a large number of traditional natural products from plants applied in allopathic medicine. According to Brantner and Grein (1994), these types of plant extracts are potential sources of antimicrobial agents, which could be used as therapeutic agents or preservatives in foods. In this sense, Das et al. (2009) evaluated the antimicrobial activity of aqueous, methanolic, and ethanolic extracts of *S. rebaudiana* leaves. All individual extracts showed potential antimicrobial activity compared to standard ampicillin, but the activities were lower than standard. However, aqueous extracts showed a high, statistically significant antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli*, and *S. typhi*. Aqueous extracts of *S. rebaudiana* exhibit high, significant antimicrobial activity not only against bacteria but also against food-spoilage fungi such as *A. solani*, *H. solani*, *A. niger*, and *P. chrysogenum* (Gosh et al., 2008) and fungi and yeast such as *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, and *Epidermophyton species*) (Jayaraman et al., 2008). Usually, the extracts are obtained at room temperature, but a higher temperature has influence on the antimicrobial activity. Khan et al. (2012) evaluated the antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* (tetracycline as a control) of *S. rebaudiana* leaf, steam, and root aqueous and ethanolic extracts obtained at 80 and 70 °C, respectively. For leaf aqueous and ethanolic extracts, the temperature increased the antimicrobial activity, although for stem and root extracts the activity decreased.

The results obtained by various authors indicate that the solubility, concentration and composition of secondary metabolites are responsible for the antimicrobial activity of the different extracts. When an aliquot of a plant extract is taken for determining of the minimum

inhibitory concentration, better susceptibility and inhibition is observed in many cases compared to the original extract. This may be due to purity of the extract, in that prior to dilution it was more viscous and unable to permeate and diffuse properly in the medium (well-diffusion assay) but, following dilution, could easily diffuse into the medium. Hence, *S. rebaudiana* can be further subjected to isolation of antimicrobial compounds. Therefore, these compounds could be proven as future potential candidates either as non-antibiotic pharmaceuticals of food preservatives and/or plant micro-biocides after proper toxicity studies in plant and animal models and clinical trials are addressed.

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Anti-inflammatory activity

Inflammation is a very complex, multifactorial, and dynamic process involving many systems, which is closely associated with the process of repair. Inflammation is defined as a local response of mammalian tissue to injury due to any agent, and manifests usually in form of painful swelling associated with some changes in skin covering the site. Inflammation can be classified either as acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is due to the increased movement of plasma and leukocytes from the blood into the injured tissues. Chronic inflammation is due to a progressive shift in the type of cells that are present at the site of inflammation, which is characterized by simultaneous destruction and healing of the tissue due to the inflammatory process [Jain et al., 2011; Loganayaki et al., 2012). According to Jain et al. (2011), carrageenan-induced paw edema is widely used for determining the acute phase of the inflammation and is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 hours after carrageenan

injection), chemical mediators such as histamine, dextran, and serotonin play a role, while in the second phase (3-4 hours after carrageenan injection), kinins and prostaglandins are involved (Ratheesh e tal 2010). The results of Arya et al. (2012) revealed that administration of methanolic extract of callus and intact plant part of *S. rebaudiana* Bertoni inhibited the edema starting from the first hour and during all phases of inflammation, which probably leads to inhibition of different aspects and chemical mediators of inflammation. It is well known that in chronic and sub-acute inflammation, ROS play an important role in modulating the extent of the inflammatory response and consequent tissue and cell injury, and antioxidants are considered as possible protective agents that reduce oxidative damage to the human body from ROS and retard the progress of many diseases. The natural phenolic, alkaloids, tannins, glycosides, and flavonoid compounds function as antioxidants by different mechanisms and, according to Arya et al. (2012), the high contents of these phytochemicals in both the extracts can explain their anti-inflammatory activity. Thus, they concluded that the methanolic extract of callus culture and intact plant part exhibited significant, dose dependent activity on the tested experimental animal models and produced a significant inhibition of carrageenan-induced paw edema in rat. The studies suggest that *S. rebaudiana* is also a potent source for phytomedicine development in the future.

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Anti-tumor activity

Cancer is a group of diseases caused by loss of cell-cycle control. Cancer is associated with abnormal, uncontrolled cell growth (Krishnamurthi, 2007). Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited

mutations, hormones, immune conditions, and mutations that occur from metabolism). Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease, and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of humankind (Divisi et al., 2006). To date, several scientific studies focused on the activity of non-nutritional compounds present in the diet, preventing the occurrence of degenerative diseases such as cancer. This heterogeneous class of molecules, generally known as phytochemicals, includes vitamins food polyphenols, such as flavonoids, phytoalexins, phenolic acids, indoles, and sulfur-rich compounds (Russo, 2007; Sporn and Suh, 2002; Surh, 2003). More than 10,000 phytochemicals have been described, and among them more than 6,000 compounds are included in the class of flavonoids. They are widely present in plant-derived foods, beverages (fruits, vegetables, and beverages such as tea, wine, beer, and chocolate), and many dietary supplements or herbal remedies (Russo et al., 2010). According to Cragg and Newman (2000), more than 50% of the drugs in clinical trials for anti-cancer properties were isolated from natural sources or are related to them. Several natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anti-cancer agents derived from plants are podophyllotoxin, taxol, vincristine, and camptothecin (Pezzuto, 1997). The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among Food and Drug Administration approved anti-cancer and anti-infectious drugs, drugs from natural origin have a share of 60 and 75%, respectively (Newman et al., 2003.). A great number of *in-vitro* and *in-vivo* methods have been developed to measure the efficiency of natural anti-cancer compounds either

as pure compounds or as plant extracts. *In-vitro* methods including the tryphan blue dye exclusion assay, LDH (lactic dehydrogenase) assay, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay, XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) assay, and sulforhodamine B assay are most commonly used for estimating anti-cancer properties of natural products from medicinal plants. Among all *in-vitro* methods, MTT and the sulforhodamine B assay are the most popular for estimating anti-cancer activity (Chanda and Nagani, 2013). However, there is a discrepancy between phytochemical concentrations applied in *in-vitro* studies (usually tens of micromolars) and those found *in vivo* (human and animal sera) after vegetable and fruit ingestion (usually below 1 μM) (Krishnamurthi, 2007). Nevertheless, this wide group of natural molecules represents a promising class of anti-cancer drugs due to their multiple targets in cancer cells and limited toxic effects on normal cells.

The anti-tumor potential of *S. rebaudiana* was evaluated *in vitro* by Jayaraman et al. (2008). These authors obtained leaf extracts with organic solvents (i.e., ethyl acetate, acetone, and chloroform) and water. These authors performed an anti-tumor assay on human laryngeal epithelioma cells (HEp2). The aqueous extract of *S. rebaudiana* showed no pronounced anti-tumor activity, but the acetone and ethyl acetate extracts were more cytotoxic to HEp2 cells. Acetone extracts showed the highest cytotoxic activity, followed by ethyl acetate and chloroform extracts. An MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was used to evaluate cytotoxicity based on metabolic reduction of MTT. In treatments with Vero (African green monkey kidney cells) cells, 1:2 and 1:4 dilutions of the acetone extract showed cytotoxicity, but there was no apparent cytotoxicity at 1:8 dilution. Further dilutions also had no

toxic effects on Vero cells. The 1:2 and 1:4 dilutions were cytotoxic to HEp2 cells, whereas the 1:8 dilution caused more than 50% cytotoxicity and also cessation of cell growth. Further dilutions had less effect on the viability of the cancerous cells. Thus, the 1:8 dilution of the acetone extract of *S. rebaudiana* is non-toxic to the normal cells and also has both anti-cancer and anti-proliferative activities against the cancerous cells.

In another study, Rajesh et al. (2010) evaluated the anti-cancer activity of an ethanolic extract of leaves of *S. rebaudiana* in rats by induced Erlischôs Ascites carcinoma. The effect of *S. rebaudiana* ethanolic extract on tumor growth by hematological parameters is presented in Table 2. The authors concluded that the ethanolic extract has shown good anti-cancer activity at the dose level of 300 mg/kg/i.p (intraperitoneal injection). The extract decreased the tumor volume, the viable and non-viable tumor cell count, and the bodyweight as well as increased the lifespan in a dose-dependent manner compared to the standard drug (5-fluoro uracil at 20 mg/kg/i.p). Studies have demonstrated the inhibitory effects of Stevia leaf extracts and their polyphenolic constituents on tumor promotion and initiation. The ethanolic extract also influenced other hematological and biochemical parameters, increased the hemoglobin level, red blood cell count, lymphocyte and monocyte counts, glucose level, protein level, and contents of urea and uric acid, and decreased the packed cell volume, neutrophils count, and cholesterol level. Stevioside, the Stevia leaf aglycones, steviol and isosteviol, and their metabolites have been reported to inhibit tumor promotion by blocking Epstein-Barr virus early antigen (EBV-EA) induction (Akihisa et al., 2004) as well as by reducing tumor formation in the two-stage mouse skin carcinogenesis model following sequential exposure to 7,12-dimethylbenz [a]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Takasaki et al., 2009). The hydrolysis product of

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stevioside, isosteviol, potently inhibits DNA replication and human cancer cell growth *in vitro* (with LD50 values of 84 to 167 εMol) (Mizushina et al., 2005). These results points to the probable anti-tumor potential of some solvent extracts of *S. rebaudiana* leaves. There is a need for further investigation of this plant in order to identify and isolate its active anti-cancer principles. The results of these studies will also need to be confirmed using in-vivo models and clinical trials.

Consumption and adverse effects

Certain acute and subacute toxicity studies indicate that Stevia and stevioside have low toxicity (Hsieh et al., 2003). Stevioside could be useful as no caloric sweetener for diabetes and phenylketonuria patients and obese persons, as no allergic reactions have been reported (Genus. 2003). However, the Food and Drug Administration (FDA) has not permitted the use of whole-leaf Stevia or crude Stevia extracts because these substances have not been approved for use as a food additive. FDA does not consider their use in food to be generally recognized as safe (GRAS) in light of reports in the literature that raise concerns about the use of these substances. Among these concerns are control of blood sugar and effects on the reproductive, cardiovascular, and renal systems (Melis and Sainati, 1991; Melis, 1999; Yodyingyuad and Bunyawong, 1991).

In this sense Shmandke (2004) observed a decreased in mean arterial pressure (9.5%) and bradycardia in healthy subjects (edge 24-40 years) after consumption (30 days) of tea made from stevia leaves. Intravenous administration of stevioside in rats there seems to be a vasodilator effect resulting in decreased mean arterial pressure and lowering of renal vascular resistance (Melis and Sainati, 1991) the authors concluded that it is possible that stevioside acts on arterial pressure and renal function as a calcium antagonist. Several studies on *S. rebaudiana* extracts

report effects on the male reproductive system, such as reduced spermatogenesis, decreased seminal vesicle weight and interstitial cell proliferation in the testes (Yamada et al., 1985) [91]. Administration of aqueous S. rebaudiana extracts (corresponding to 0.667 g dried leaves/mL, 2 mL/rat twice a day) for 60 days to the rat decreased seminal vesicle weight by about 60% (Mori et al., 1981). Older studies reported anti-fertility effects, as well as decreases in the weights of the testes, seminal vesicle, and cauda epididymides and a reduction in spermatozoa concentration, in rats administered crude stevia extracts. However, in most old studies of reproduction performance the administered dose has been low and not comparable to those used in other toxicological studies. Furthermore, the administered stevioside extracts have chemically not been adequately described.

In a recent study, Curry and Roberts (2008) found no treatment-related effects of rebaudioside A on mating performance, fertility, gestation lengths, and estrous cycle in rats in a two-generation study. Those investigators dosed rats with 0, 7,500, 12,500, and 25,000 ppm rebaudioside A via the diet. Female rats in the 12,500 ppm and 25,000 ppm groups and male rats in the 25,000 ppm group of the first generation and male and female rats in the 25,000 ppm group of the second generation showed significant decreases in body weight gains compared to controls. The investigators considered those effects to be toxicologically insignificant due to the lack of adverse effects on those animals survival, condition of their offspring, their pre-weaning reflex development, weight gain after 25 days, and timing of sexual maturation. In other study Curry et al. (2008) concluded that steviol glycosides do not poses a reproductive or developmental hazard.

Conclusions

The sweet herb of Paraguay, *S. rebaudiana,* is fast becoming a source of high-potency sweetener, which produces a sweet taste but has no calorific value. Many research studies on its chemical and biological properties have been done recently. Studies have reported the health-promoting properties of stevia, which is well known as a therapeutic agent. Exhaustive work has been done on the plant but there is still a need for research work on the pharmacological aspects of rebaudioside, adverse effects, and ADME (absorption, distribution, metabolism and excretion) studies of stevioside on humans. Because the safety of whole-leaf or crude Stevia extracts for human consumption remains controversial, there is a clear need for further experimentation with respect to the metabolic fate of steviol glycosides and to clarify the risk towards genotoxicity.

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Tables

Table 1. Antioxidant activity of *S. rebaudiana* Bertoni from Bangladesh (Ghanta et al., 2007).

Extraction solvent (leaves)	DPPH free radical scavenging activity (IC_{50})	Extraction solvent (leaves)	Reducing power test (Absorbance)
Ethanol (80%)	8.02 ± 0.874	Ethanol (80%)	1.0717 ± 0.0017
1-butanol	23.60 ± 0.763	n-hexane	0.5684 ± 0.0013
Water	43.81 ± 0.459	Dichloromethane	0.8191 ± 0.0017
Methanol	44.61 ± 0.821	1-butanol	0.9819 ± 0.0014
Hot water	23.70 ± 0.861	Water	1.5552 ± 0.0015
Ascorbic acid	4.21 ± 0.861	Hot n-hexane	0.5894 ± 0.0014
		Hot dichloromethane	0.9498 ± 0.0015
		Hot methanol	0.9086 ± 0.0018
		Hot water	0.9972 ± 0.0029
		Ascorbic acid	1.3741 ± 0.0031

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