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Review

Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects

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ABSTRACT

Stevia rebaudiana Bertoni, an ancient perennial shrub of South America, produces diterpene glycosides that are low calorie sweeteners, about 300 times sweeter than saccharose. Stevia extracts, besides having therapeutic properties, contain a high level of sweetening compounds, known as steviol glycosides, which are thought to possess antioxidant, antimicrobial and antifungal activity. Stevioside and rebaudioside A are the main sweetening compounds of interest. They are thermostable even at temperatures of up to 200 °C, making them suitable for use in cooked foods. *S. rebaudiana* has a great potential as a new agricultural crop since consumer demand for herbal foods is increasing and proximate analysis has shown that *Stevia* also contains folic acid, vitamin C and all of the indispensable amino acids with the exception of tryptophan. Stevia cultivation and production would further help those who have to restrict carbohydrate intake in their diet; to enjoy the sweet taste with minimal calories.

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1. Introduction

Stevia rebaudiana Bertoni is a branched bushy shrub of the Asteraceae family, native to the Amambay region in the north east of Paraguay. It also occurs in the neighbouring parts of Brazil and Argentina (Soejarto, 2002). Today its cultivation has spread to other regions of the world, including Canada and some parts of Asia and Europe (Amzad-Hossain, Siddique, Mizanur-Rahman, & Amzad-Hossain, 2010; Gardana, Simonetti, Canzi, Zanchi, & Pietta, 2003). Presently, *Stevia* is well-known for its high content of sweet diterpene (about 4–20%) in dry-leaf matter (Ghanta, Banerjee, Poddar, & Chattopadhyay, 2007). It is the source of a number of sweet *ent*-kaurene diterpenoid glycosides (Prakash, Dubois, Clos, Wilkens, & Fosdick, 2008), and the *stevia* glycosides are the compounds responsible for the sweet taste. Among the 230 species in the genus *Stevia*, only the species *rebaudiana* and *phlebophylla* produce steviol glycosides (Brandle & Telmer, 2007).

S. rebaudiana Bertoni (Fig. 1) was botanically classified in 1899 by Moisés Santiago Bertoni, who described it in more detail. Initially called *Eupatorium rebaudianum*, its name changed to *S. rebaudiana* (Bertoni) Bertoni in 1905. The sweet principle was first isolated in 1909 and only in 1931 was the extract purified to produce stevioside, the chemical structure of which was established in 1952 as a diterpene glycoside. Stevioside is described as a glycoside comprising three glucose molecules attached to an aglycone, the steviol moiety. During the 1970s, other compounds were isolated, including rebaudioside A, with a sweetening potency even higher than stevioside (Barriocanal et al., 2008).

Steviol is the common aglycone backbone of the sweet stevia glycosides that have been analyzed by liquid chromatography coupled with UV, MS and ELS detection (Cacciola et al., 2011). Stevioside, one of the stevia glycosides, is about 300 times sweeter than saccharose and can be particularly beneficial to those suffering from obesity, diabetes mellitus, heart disease and dental caries (Ghanta et al., 2007).

Although *Stevia* continues to be a rare plant in its native habitat, agricultural production in South America and Asia, and ornamental use in Europe and North America have made its occurrence in the world perhaps more common than it ever was in the past (Brandle & Telmer, 2007). Studies revealed that *Stevia* has been used since ancient times for various purposes throughout the world (Goyal, Samsher, & Goyal, 2010). For centuries, the Guarani tribes of Paraguay and Brazil used *Stevia* species, primarily *S. rebaudiana*, which they called *ka'a he'ê* ("sweet herb"), as a sweetener in yerba mate and medicinal teas for treating heartburn and other ailments (Brandle & Telmer, 2007). *S. rebaudiana* Bertoni has attracted economic and scientific interests due to the sweetness and the supposed therapeutic properties of its leaf. Japan was the first country in Asia to market stevioside as a sweetener in the food and drug industry. Since then, cultivation of this plant has expanded to other countries in Asia, including China, Malaysia, Singapore, South Korea, Taiwan, and Thailand (Chatsudthipong & Muanprasat, 2009). *Stevia* and stevioside have been applied as substitutes for saccharose, for treatment of diabetes mellitus, obesity, hypertension and caries prevention (Pól, Hohnová, & Hyötyläinen, 2007), and a number of studies have suggested that, besides sweetness, stevioside, along with related compounds which include rebaudioside A, steviol and isosteviol, may also offer therapeutic benefits, as they have anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumour, anti-diarrhoeal, diuretic, and immunomodulatory effects (Chatsudthipong & Muanprasat, 2009). The leaves of *Stevia* has functional and sensory properties superior to those of many other high-potency sweeteners, and is likely to become a major source of high-potency sweetener for the growing natural food market in the future (Goyal et al., 2010).

Fig. 1. *Stevia rebaudiana* Bertoni leaves.

Toxicological studies have shown that stevioside does not have mutagenic, teratogenic or carcinogenic effects. Likewise, allergic reactions have not been observed when it is used as a sweetener (Pól, Hohnová, et al., 2007). Recently completed studies on the general and reproductive toxicity of rebaudioside A corroborate studies carried out with purified steviol glycosides, demonstrated its safety at high dietary intake levels. Comparative metabolism studies provide further affirmation of the common metabolic pathway for all steviol glycosides and the common metabolism between rats and humans (Carakostas, Curry, Boileau, & Brusick, 2008).

The purpose of this review is to bring together a selection of essential basic data coming from numerous scientific researches on stevia, a naturally occurring sweetener. Emphasis was placed on the remarkable potential of stevia as an intense high-potency sweetener together with its functional and health-promoting properties, making thereby a contribution in enhancing the importance of *S. rebaudiana* as a promising new agricultural crop. This may contribute to satisfy today's need for food ingredients of low-calorie with nutritional, therapeutic and functional properties. Consumers' demand for herbal foods may encourage *Stevia* cultivation and production and may help those who have to restrict carbohydrate intake or reduce the glycemic index in the diet, to enjoy the sweet taste with minimal calories. This review also aims for a better understanding and acceptance of stevia as a natural raw material for the health food industry.

2. Botanical description

Stevia is a genus of about 200 species of herbs and shrubs in the sunflower family (Asteraceae). It grows up to 1 m tall (Mishra, Singh, Kumar, & Prakash, 2010). The plant is a perennial herb with an extensive root system and brittle stems producing small, elliptic leaves (Shock, 1982). The leaves are sessile, 3–4 cm long, elongate-lanceolate or spatulate shaped with blunt-tipped lamina, serrate margin from the middle to the tip and entire below. The upper surface of the leaf is slightly granular pubescent. The stem is woody and weak-pubescent at the bottom. The rhizome has slightly branching roots. The flowers are pentamerous, small and white with a pale purple throat. They are composite surrounded by an involucre of epicalyx. The capitula are in loose, irregular, sympodial cymes. The tiny white florets are borne in small corymbs of 2–6 florets, arranged in loose panicles. The fruit is a five-ribbed spindle-shaped achene (Blumenthal, 1996; Katayama, Sumida, Hayashi, & Mitsuhashi, 1976).

Stevia will grow well on a wide range of soils given a consistent supply of moisture and adequate drainage; plants under cultivation can reach up to 1 m or more in height (Shock, 1982). It is cultivated as a perennial shrub in subtropical regions including parts of the United States. The plant is indigenous to the northern regions of South America and grows wild in the Highlands of Amambay and near the source of the river Monday (a border area between Brazil and Paraguay). It is being cultivated in continental China, Taiwan, Thailand, Korea, Brazil, and Malaysia. Besides the above-mentioned countries, *Stevia* is also grown in Israel, the Ukraine, the UK, the Philippines, Canada, Hawaii, California and all over South America (Sivaram & Mukundam, 2003).

Stevia must be cultivated as an annual plant in mid- to high-latitude regions, where longer days favour leaf yield and stevioside contents. Oddone (1997) considers *Stevia* to be self-incompatible and insect pollinated. Additionally, he considers "clear" seeds to be infertile. Seeds are contained in slender achenes, about 3 mm in length. Each achene has about 20 persistent pappus bristles. Poor seed germination is one of the factors limiting large-scale cultivation. Carneiro, Muniz, and Guedes (1997), Duke (1993) and Shock (1982) reported poor percentages of viable seeds in *Stevia*. Consequently, propagation is a special concern for northern growers who must grow *Stevia* as an annual crop. Propagation by seeds does not allow the production of homogeneous populations, resulting in great variability in important features like sweetening levels and composition (Nakamura & Tamura, 1985; Tamura, Nakamura, Fukui, & Tabata, 1984). *Stevia* is therefore usually propagated by stem cuttings which root easily, but require high labour inputs. The vegetative propagation is further limited by the lower number of individuals that can be obtained simultaneously from a single plant. Due to the above-mentioned difficulties, tissue culture would be the best alternative for rapid mass propagation of *Stevia* plants (Sivaram & Mukundam, 2003).

Stevia suffers from the cold and does not usually tolerate temperatures below 9 °C. However, it occasionally tolerates temperatures near to zero. For rapid growth, 20–24 °C are necessary (Singh & Rao, 2005). On the other hand, *Stevia* has a remarkable water need, the leaves and stems can wilt rapidly, but also recover rapidly if the stress is not prolonged; this is a limitation to the area suitable for its cultivation. It grows fast and can be grown as an annual herb during late spring and summer. Accordingly, *Stevia* could become an interesting and profitable new crop for the tropics (as a perennial herb), for warm areas including temperate areas with hot and rainy summers (as an annual summer crop) and for large parts of the Mediterranean, again as annual crop during spring and autumn or irrigated as a perennial.

Stevia can be grown in relatively poor soil. The plants can be used for commercial production for 8 years at the stretch of which harvests of vegetative parts takes place six times a year. The roots remained in place and the plant regenerates rapidly. The quantity of dry leaves that can be harvested varies from 15 to 35 g per plant (Mishra et al., 2010). According to Serio (2010), one planted hectare can produce between 1000 and 1200 kg of dried leaves that contain 60–70 kg stevioside, which is a low yield compared to sugar cane or sugar beet. However, 70 kg stevioside, which is 300 times sweeter than saccharose, is equivalent to a yield of 21,000 kg sugar per hectare.

There are about 90 varieties of *S. rebaudiana* developed all around the world depending upon the different climatic requirements (Ibrahim, Nasr, Mohammed, & El-Zefzafi, 2008; Singh & Rao, 2005). The land sites are ploughed and/or cultivated twice to prepare a fairly smooth firm-planting surface. Transplants from cuttings are superior to propagation from seeds that are placed in plug trays in the green house for a period of 7–8 weeks, a rather expensive process. *Stevia* plug plants are then planted into the field on either 53 cm or 61 cm row spacing with a total plant density on the order of 100,000 plants per hectare. However, different climatic conditions would influence *Stevia* cultivation, so it is advisable to carry out trials in each planting zone to establish adequate plant population density for that particular area (Rahmesh, Singh, & Megeji, 2006). The *Stevia* plants appear to have low nutrient requirements; generally, the plant requires frequent shallow irrigation. Normally, irrigation is applied at least one time per week, if the stem tips are drooping (Kaushik, Pradeep, Vamshi, Geetha, & Usha, 2010).

3. Biochemical and nutritional aspects of *Stevia*

Savita, Sheela, Sunanda, Shankar, and Ramakrishna (2004) analysed *Stevia* leaves on a dry weight basis and calculated an energy value of 2.7 kcal g^{-1}. This means that *Stevia* may be granted the status of a low calorie sweetener, since its sweetness is intense and comparable to that of other commercial sweeteners. Intense sweeteners include acesulfame K (calorie-free), aspartame (4 kcal g^{-1}), saccharin (calorie-free) and sucralose (calorie-free) (Savita et al., 2004). Calorie contribution to the diet by the commonly used saccharose, which is considered high since it is metabolised completely by the body, has a potential to escalade towards overweight status. In this context, the use of *Stevia* as a low-calorie sweetener could be of immense help in restricting or controlling calorie intake in the diet.

3.1. Functional properties of Stevia leaf powder

According to Mishra et al. (2010) *Stevia* leaf presents values of bulk density of 0.443 g ml^{-1}, water holding capacity of 4.7 ml g^{-1}, fat absorption capacity of 4.5 ml g^{-1}, emulsification value of 5.0 ml g^{-1}, swelling index of 5.01 g g^{-1}, solubility of 0.365 g g^{-1} and pH of 5.95.

Bulk density of *Stevia* leaf powder appeared to be low in comparison to protein-rich pulses. Higher bulk densities are usually desirable for the purpose of reducing paste thickness, an important factor in child feeding where bulk is of concern. However, *Stevia* leaf powder appears to lack this property. On the other hand, the study of Mishra et al. (2010) showed an increased water holding capacity of the *Stevia* leaf powder, which appears to be advantageous and may be due to high protein content. Proteins would increase water holding capacity, thus enhancing the swelling ability, an important function of protein in preparation of viscous foods such as soups, gravies, dough and baked products. The ability of protein to aid the formation and stabilization of emulsion is also critical in many foods applications, such as cake, batters, coffee whiteners, milks, frozen desserts and others. This property depends heavily on composition and stress under which the product is subjected during processing (Savita et al., 2004). Fat absorption capacity has been attributed to the physical entrapment of oil. *Stevia* leaf powder seems to possess an adequate fat absorption capacity, allowing it to play an important role in food processing, since fat acts on flavour retainers and increases mouthfeel of foods. Crammer and Ikan (1986) affirmed that since stevioside is stable at 95 °C it is a suitable sweet additive for cooked or baked foods. The leaves, as well as the pure stevioside extracts, can be used in their natural state or cooked, and are thermostable at temperature up to 200 °C (Serio, 2010). Incubation of the solid sweetener stevioside at elevated temperatures for 1 h showed good stability up to 120 °C, whilst at temperatures exceeding 140 °C forced decomposition was seen which resulted in total decomposition by heating at 200 °C (Abou-Arab, Abou-Arab, & Abu-Salem, 2010). Chang and

Table 1
Proximate analysis of dried *Stevia* leaves (g 100 g^{-1} dry weight basis).

Component	References						
	Mishra et al. (2010)	Goyal et al. (2010)	Serio (2010)	Savita et al. (2004)	Abou-Arab et al. (2010)	Tadhani and Subhash (2006a)	Kaushik et al. (2010)
Moisture	7	4.65	ND	7	5.37	ND	7.7
Protein	10	11.2	11.2	9.8	11.40	20.4	12
Fat	3	1.9	5.6	2.5	3.73	4.34	2.7
Ash	11	6.3	ND	10.5	7.41	13.1	8.4
Carbohydrate	52	ND	53	52	61.9	35.2	ND
Crude fibre	18	15.2	15	18.5	15.5	ND	ND

ND, not determined.

Cook (1983) reported that Stevia sweeteners have high heat stability after 1 h heating at 100 °C. Besides, it was also reported that stevioside and rebaudioside A are reasonably thermally stable under the elevated temperatures used in food processing and do not undergo browning or caramelization when heated (Abou-Arab et al., 2010).

3.2. Carbohydrates

Carbohydrates perform numerous essential roles in living beings. Thus, monosaccharides are the major source of energy in human metabolism, while polysaccharides serve as the storage of energy and can act as structural components. Other beneficial health effects have also been linked to these compounds. This includes a prebiotic effect as well as other less common antioxidant or anti-inflammatory activities (Bernal, Mendiola, Ibáñez, & Cifuentes, 2011). The benefits associated to *Stevia* leaf are mainly due to their nutritional composition (Table 1), which is a good source of carbohydrates, protein and crude fibre, that promotes wellness and reduces the risk of certain diseases. In *S. rebaudiana* roots and leaves, inulin-type fructooligosaccharides, a naturally occurring plant polysaccharide with important functional properties related to prebiotics, dietary fibre, role lipid metabolism and diabetes control, have been isolated by Braz de Oliveira et al. (2011). They obtained from the roots and leaves of the plant a yield of purified fructooligosaccharides of 4.6% and 0.46%, respectively. This indicates a possible application of extracts as a dietary supplement (Braz de Oliveira et al., 2011).

3.3. Proteins

Proteins, peptides and/or amino acids are found in a great variety of matrices including animals, fungi, vegetables, cereals, etc. (Bernal et al., 2011). Proteins are molecules composed of amino acids necessary for growth and repair of body tissues. Their importance lies mainly in that they are an essential constituent of cells and need to be replaced over time, which makes protein intake indispensable. To determine the protein quality of a food it is necessary to know the total protein content as well as the kinds of amino acids present, especially the content of the essential amino acids (Latham, 2002). Mohammad, Mohammad, Sher, Habib, and Iqbal (2007) identified nine amino acids in *Stevia* leaves, namely glutamic acid, aspartic acid, lysine, serine, isoleucine, alanine, proline, tyrosine and methionine. Abou-Arab et al. (2010) found still more amino acids in the *Stevia* leaves. Altogether seventeen amino acids were determined and classified as essential and non-essential amino acids, unfortunately including arginine as one of the indispensable amino acids (Table 2). According to the report of a joint FAO/WHO/UNU Expert Consultation (WHO, 2007), the indispensable amino acids are leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine and histidine. The daily requirements of these amino acids in human nutrition are also summarized in Table 2. This shows that *Stevia* leaves contained almost all of the indispensable amino acids, including tyrosine and cysteine. Only the amino acid tryptophan is missing. This means that after extraction of stevioside from the leaves, the residue could be a valuable source of indispensable amino acids for health products. Their content can match the protein requirements recommended by the World Health Organization (WHO, 2007).

3.4. Minerals

Minerals have many important functions in the human body. Some mineral elements are needed only in very small amounts in human diets, but are vital for metabolic purposes, and are thus called essential trace elements (Latham, 2002). The elements considered essential or required for the normal functioning of the body, are classified according to their relative amounts or

Table 2
Amino acid composition of *Stevia rebaudiana* leaves and summary of adult indispensable amino acid requirements.

Reported by Abou-Arab et al. (2010)				Report of a Joint WHO/FAO/UNU Expert Consultation (WHO, 2007)		
Essential amino acid g 100 g^{-1} d.m.		Non-essential amino acid g 100 g^{-1} d.m.		Indispensable amino acid	Report of (2002) mg/kg per day	Report of (1985) mg/kg per day
Arginine[a]	0.45	Aspartate	0.37	Histidine	10	8–12
Lysine	0.70	Serine	0.46	Isoleucine	20	10
Histidine	1.13	Glutamic	0.43	Leucine	39	14
Phenyl alanine	0.77	Proline	0.17	Lysine	30	12
Leucine	0.98	Glycine	0.25	Methionine + cysteine	15	13
Methionine	1.45	Alanine	0.56	Phenylalanine + tyrosine	25	14
Valine	0.64	Cysteine[b]	0.40	Threonine	15	7
Threonine	1.13	Tyrosine[b]	1.08	Tryptophan	4	3.5
Isoleucine	0.42			Valine	26	10
Total	7.67	Total	3.72	Total	184	93.5

[a] Not considered as indispensable amino acid in Technical Report FAO/WHO/UNU (WHO, 2007).
[b] Considered indispensable under specific situation.

Table 3
Minerals content (mg 100 g^{-1}) of dried *Stevia* leaves.

Minerals	References					
	Mishra et al. (2010)	Goyal et al. (2010)	Serio (2010)	Tadhani and Subhash (2006a)	Kaushik et al. (2010)	Abou-Arab et al. (2010)
Calcium	464.4	544	600	1550	722	17.7
Phosphorous	11.4	318	318	350	ND	ND
Sodium	190	89.2	ND	160	32.7	14.93
Potassium	1800	1780	1800	2510	839	21.15
Iron	55.3	3.9	3.9	36.3	31.1	5.89
Magnesium	349	349	500	ND	ND	3.26
Zinc	1.5	1.5	ND	6.39	ND	1.26

ND, not determined.

requirements. The main elements are sodium, magnesium, phosphorus, sulphur, chlorine, potassium, and calcium which are classified as macronutrients and the minor elements, considered micronutrients, are chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine (Adotey, Serfor-Armah, Fianko, & Yeboah, 2009; Szefer & Nriagu, 2007). The presence of macro and micronutrients in foods is important for the development and maintenance of vital body functions. They are involved in all aspects of growth, health and reproduction, participating also in the formation of cells, tissues and organs (Szefer & Nriagu, 2007). *Stevia* contains substantial amounts of these important nutrients, which further establishes it as a mineral loaded ingredient needed to protect the body, regulate and maintain the various metabolic processes. Potassium, calcium, magnesium, and sodium which are nutritionally important, were found in reasonable amount in *Stevia* leaves. The high concentration of these minerals would be very beneficial to health (Choudhary & Bandyopadhyay, 1999). As reported by some authors, the mean concentrations of macro and micro elements that have been determined in dried *Stevia* leaves are shown in Table 3. The high content of potassium determined in all studies is remarkable, although the amount of potassium found by Abou-Arab et al. (2010) seems to be very low compared to that of the other studies, which may be explained by different growth conditions, as described by Rahmesh, Singh, and Megeji (2006).

Zinc and iron are found in foods of plant and animal origin and are present in *Stevia* leaves. According to Wu et al. (2005), zinc is a mineral that acts as a non-enzymatic antioxidant, so that its consumption would help in preventing oxidative damage of the cell. The main biological function of iron is the transport of oxygen to the body and consequently a lack of this mineral in the diet leads to anaemia. The high amount of iron in *Stevia* leaves could again be helpful in contributing to the maintenance of a normal haemoglobin level in the body. Furthermore, *Stevia* leaves could also be used to prepare various sweet preparations to combat iron deficiency in anaemia which is a major nutritional disorder in developing countries (Abou-Arab et al., 2010).

3.5. Lipids

Lipids are a large group of natural compounds. Their main biological functions include energy storage, structural components of cell membranes and important signalling molecules. Although humans and other mammals use various biosynthetic pathways to both break down and synthetize lipids, some essential lipids cannot be made in this way and must be obtained from diet. Interestingly, many papers have discussed the health benefits that can be derived from some of these lipids (Bernal et al., 2011). Fatty acids are carboxylic acids with a variable unbranched aliphatic tail (chain), which is either saturated or unsaturated. They are important as nutritional substances in living organisms. Long-chain polyunsaturated fatty acids (PUFA), especially those of the *n*-3 series, such as α-linolenic acid (18:3 *n*-3), are essential for human metabolism and have many beneficial effects including the prevention of a number of diseases, such as coronary heart diseases, inflammation, autoimmune disorders, hypertension, hypotriglyceridemic effects (Bernal et al., 2011). Linolenic acid, which is as healthy as the linoleic acid, is considered an essential fatty acid (EFA) necessary for good health. EFAs are important in the synthesis of many cellular structures and several biologically important compounds (Latham, 2002). Moreover, other polyunsaturated fatty acids are essential for the human body, performing many functions such as maintenance of cell membranes and production of prostaglandins (regulators of many body processes, including inflammation and blood clotting). Fats are also needed in the diet as input for fat-soluble vitamins in foods (A, D, E and K) and can be absorbed to regulate cholesterol metabolism (Pinazo-Durán, Zanón-Moreno, & Vinuesa-Silva, 2008). In the leaf oil of *Stevia*, Tadhani and Subhash (2006a) identified six fatty acids (Table 4) using methyl ester standards. Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were identified in the leaf oil. Among the identified fatty acids, palmitic acid content was found to be highest, whereas stearic acid content was least. Stevia leaf oil proves to be a rich source of linolenic acid. This high value of linolenic acid may contribute to maintain an ideal fatty acid ratio in human diet.

Table 4
Fatty acid composition (g 100 g^{-1}) of *Stevia* leaf oil (Tadhani & Subhash, 2006a).

Fatty acids	g 100 g^{-1}
Palmitic acid (C16)	27.51
Palmitoleic acid (C16-1)	1.27
Stearic acid (C18)	1.18
Oleic acid (C18-1)	4.36
Linoleic acid (C18-2)	12.40
Linolenic acid (C18-3)	21.59

3.6. Vitamins

Vitamins are organic substances present in very small quantities in food, but necessary for metabolism. They are grouped together not because they are chemically related or have similar physiological functions, but because they are vital factors in the diet and they all were discovered in connection with the diseases that were caused owing to their deficiency (Latham, 2002). They are classified as either water-soluble or fat soluble. There are 13 vitamins: 4 fat-soluble (A, D, E and K) and 9 water-soluble (8 vitamins of the B group and vitamin C). These compounds have diverse biochemical roles. Some have hormone-like functions as regulators of mineral metabolism (e.g., vitamin D), or regulators of cell and tissue growth and differentiation (e.g., some forms of vitamin A). Others work as antioxidants (e.g., vitamin E and sometimes vitamins B and C). The largest numbers of vitamins (e.g. B complex

Table 5
Water soluble vitamins of *S. rebaudiana* leaf and callus extracts (mg 100 g^{-1} dry base of extract) (Kim et al., 2011).

Vitamin	Leaf	Callus
Vitamin C	14.98 ± 0.07	1.64 ± 0.02
Vitamin B2	0.43 ± 0.02	0.23 ± 0.02
Vitamin B6	0.00 ± 0.00	0.00 ± 0.00
Folic acid	52.18 ± 0.21	0.09 ± 0.01
Niacin	0.00 ± 0.00	0.00 ± 0.00
Thiamin	0.00 ± 0.00	0.00 ± 0.00

Table 6
Amount of sweet glycosides in *Stevia* leaves.

Glycosides	Contents, % of the leaves dry weight		
	Gardana et al. (2010)	Goyal et al. (2010)	Kinghorn and Soejarto (1985)
Stevioside	5.8 ± 1.3	9.1	5–10
Rebaudioside A	1.8 ± 1.2	3.8	2–4
Rebaudioside C	1.3 ± 1.4	0.6	1–2
Dulcoside A	ND	0.3	0.4–0.7

ND, not determined.

vitamins) work as precursors of enzyme cofactors (Bernal et al., 2011). The protective effects of plant products are due to the presence of several components that have distinct mechanisms of action; some are enzymes and proteins, and others are low molecular weight compounds like vitamins (Halliwell, Gutteridge, & Arurma, 1987). It has been reported that the levels of plasma antioxidant vitamins and minerals such as vitamin C, E, folic acid, and zinc declined as oxidative damage increased in stressed animals (Sahin, Kucuk, Sahin, & Sari, 2002).

Kim, Yang, Lee, and Kang (2011) studied the amounts of water-soluble vitamins in the *Stevia* leaf and callus extracts (Table 5), and determined that the contents of folic acid, vitamin C and vitamin B2 in the leaf extracts were significantly higher than those of the callus extracts. In the leaf extract, folic acid was found to be the major compound, followed by vitamin C. In the callus extract, vitamin C was the major compound, followed by vitamin B.

4. Phytochemical constituents

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins and polyphenols (Edeoga, Okwu, & Mbaebie, 2005). *S. rebaudiana* (commonly referred to as honey leaf, candy leaf and sweet leaf) is rich in terpenes and flavonoids. The phytochemicals present in *S. rebaudiana* are austroinullin, β-carotene, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol, stevioside and thiamine (Jayaraman, Manoharan, & Illanchezian, 2008).

4.1. Diterpene glycosides

Glycosides are compounds containing a carbohydrate molecule (sugar) bound to a non-carbohydrate moiety. These compounds are mainly found in plants, and they can be converted, by hydrolytic cleavage, into a sugar and a non-sugar component (aglycone). They are named specifically by the type of sugar that they contain, as glucosides (glucose), pentosides (pentose), fructosides (fructose), etc. (Bernal et al., 2011).

Stevia, the common name for the extract stevioside from the leaves of *S. rebaudiana* Bertoni, is a new promising renewable raw food stuff on the world market and is a natural, sweet-tasting calorie-free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners (Anton et al., 2010; Das, Dang, & Shivananda, 2006). The natural sweeteners of *Stevia* leaves, called steviol glycosides, are diterpenes, isolated and identified as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside (Geuns, 2003).

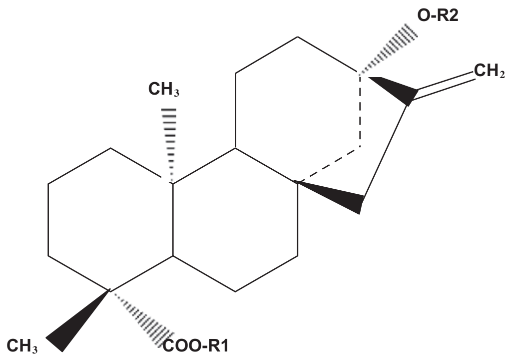
Stevioside was reported to be the most abundant stevia glycoside (4–13% w/w) found in the plant leaves. It is followed by rebaudioside A (2–4% w/w), rebaudioside C (1–2% w/w) and dulcoside A (0.4–0.7% w/w) (Makapugay, Nanayakkara, & Kinghorn, 1984). Steviolbioside, rebaudioside B, D, E and F were also identified in the leaf extracts, but as minor constituents (Geuns, 2003). In addition to these compounds, *Stevia* extracts were also reported to contain flavonoids, sterebins A to H, triterpenes, volatile oil components, pigments, gums and inorganic constituents (Geuns, 2003).

The glycosides found mainly in the leaves of the plant, make up to 15% of the content, depending on variety (Giraldo, Marín, & Habeych, 2005). Contents of the sweet glycosides in the leaves of *Stevia* are shown in the Table 6. Its amount depends on growing conditions (Pól et al., 2007), as well as on the adoption of modern agronomical techniques (Geuns, 2003; Nepovim, Drahosova, Valicek, & Vanek, 1998). The content of rebaudioside B is negligible in comparison to that of stevioside (Pól et al., 2007). Conversely, purified extracts obtained from *Stevia* leaves and offered on the market contain mainly stevioside (>80%) or rebaudioside A (>90%) (Gardana, Scaglianti, & Simonetti, 2010).

Work to elucidate the chemical structures of *S. rebaudiana* sweeteners began in the early 20th century, but proceeded slowly. The structures of stevioside and rebaudioside were not fully determined until 1960. During the 1970s, additional sweet components, including rebaudiosides A–E, were isolated from *S. rebaudiana* leaves and characterized by Osamu Tanka and co-workers at Hiroshima University in Japan (Kinghorn & Soejarto, 1985). However, some evidence exists that rebaudioside B and steviolbioside are not native constituents of *S. rebaudiana*, but are formed by partial hydrolysis during extraction (Prakash et al., 2008), being thus artifacts of the extraction procedure (Kennelly, 2002).

Stevioside has the chemical formula of a diterpene glycoside ($C_{38}H_{60}O_{18}$) and as an active component in *Stevia* leaves is responsible for the edulcorant properties. Its use has been approved in Brazil, Argentina and Paraguay as well as in China, Korea and Japan. These molecules are highly stable in aqueous solutions within a broad range of pH and temperature (Abou-Arab et al., 2010; Virendra & Kalpagam, 2008). Steviosides show remarkable stability in aqueous solution over a wide range of pH values and temperatures. Under thermal treatment in a pH range of 1–10 over 2 h at 60 °C, hardly any degradation of stevioside was be observed, only slight losses up to 5% (pH 2 and 10) were determined on heating to a temperature of 80 °C. Under strong acidic conditions (pH 1.0) forced decomposition of stevioside was observed which resulted in total decomposition after incubation at a temperature of 80 °C for 2 h (Abou-Arab, Abou-Arab, & Abu-Salem, 2010). Similar results were reported by Buckenhuskers and Omran (1997) who showed that the stevioside possess an excellent heat stability is up to 100 °C for 1 h at pH range 3–9, but rapid decomposition occurs at pH level greater than 9 under these conditions.

All diterpene glycosides isolated from *S. rebaudiana* leaves have the same steviol backbone (Fig. 2) and differ mainly in the content of carbohydrate residues (R1 and R2), mono-, di-, and trisaccharides containing glucose and/or rhamnose at positions C13 and C19 (Kochikyan, Markosyan, Abelyan, Balayan, & Abelyan, 2006). The sweetness of rebaudiosides increases with increasing amount of sugar units bonded to the steviol aglycone. However, their content in the plant material decreases at the same time (Kovylyaeva



Compound	R1	R2
Steviol	H	H
Steviolbioside	H	β-Glc-β-Glc(2→1)
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) I β-Glc(3→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) I β-Glc(3→1)
Rebaudioside C (Dulcoside B)	β-Glc	β-Glc-α- Rha(2→1) I β-Glc(3→1)
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) I β-Glc(3→1)
Rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
Rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) I β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α- Rha(2→1)

Fig. 2. Structure of the major glycosides of *Stevia rebaudiana* leaves. Glc, Xyl, and Rha represent, respectively, glucose, xylose, and rhamnose sugar moieties (Geuns, 2003).

et al., 2007). The edulcorant properties of those glycosides, however, differ from one another. Rebaudioside A, for example, which has an extra glucose unit relative to stevioside, is superior in terms of both sweetness and quality of taste. Pure stevioside usually produces a significant bitter aftertaste (de Oliveira, Packer, Chimelli, & de Jesus, 2007).

The sweetness of any of the stevia compounds is greater than that of saccharose: rebaudioside A (250–450 times); rebaudioside B (300–350 times); rebaudioside C (50–120 times); rebaudioside D (250–450 times); rebaudioside E (150–300 times); dulcoside A (50–120 times); and steviolbioside (100–125 times). On average, the sweetness of the steviol glycosides is 250–300 times greater than that of saccharose, with low water solubility and high melting points (Crammer & Ikan, 1987). Stevioside, the most abundant steviol glycoside in the leaf of the plant, has become well known for its intense sweetness (250–300 times sweeter than solutions containing 0.4% saccharose), and is used as a non-caloric sweetener in several countries (Chatsudthipong & Muanprasat, 2009; Gardana et al., 2010).

5. Other constituents

The presence of biologically important secondary plant products in *Stevia* leaf contributes to its medicinal value, since they exhibit physiological activity (Sofowara, 1993). These secondary plant constituents include labdanes, flavonoids, sterols, triterpenoids, chlorophylls, organic acids, mono-disaccharides, and inorganic salts (Gardana et al., 2010). Tadhani and Subhash (2006a) subjected leaves of *S. rebaudiana* to qualitative phytochemical screening for the identification of various classes of active chemical constituents. The powdered leaf subjected to preliminary phytochemical screening using chemical methods showed the most abundant compounds in the leaf extract to be tannins and alkaloids, followed by cardiac glycosides, saponins, sterols and triterpenes, reducing compounds and anthraquinones. Tests for cyanogenetic glycosides, however, showed negative results.

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga et al., 2005).

Savita et al. (2004) found a high percentage of anti-nutritional factors in extracts of *Stevia* leaf dissolved in water: oxalic acid and tannins, 2295.0 and 0.010 mg/100 g (dry wt. basis), respectively. Oxalic acid may hinder the bio availability of calcium, iron and other nutrients as in the case of green leafy vegetables. Tannins have been reported to have several pharmacological activities such as spasmolytic activity in smooth muscle cells (Tona et al., 1999). It has also been reported that they have free radical scavenging properties (Bharani, Ganguly, & Bhargava, 1995) and antioxidant activity. Saponins, which are amphipathic glycosides, have also been studied in seeds, plants and cereals. Saponins can stimulate muscle growth and raise testosterone levels and they can also show anti-bacterial, immunological and anti-diabetic properties (Bernal et al., 2011).

6. Extraction and determination of steviol glycosides

The different techniques used to obtain steviol glycosides can be classified in various categories, those based on solvent extraction (Bondarev, Nosov, & Reshetnyak, 2001; Morita, Fujita, & Iwamura, 1978), chromatographic adsorption (Ahmed & Dobberstein, 1982; Kolb, Herrera, Ferreyra, & Uliana, 2001; Makapugay et al., 1984; Striedner, Czygan, & Braunegg, 1991), ion exchange (Fuh & Chiang, 1990; Giovanetto, 1990; Payzant, Laidler, & Ippolito, 1999) selective precipitation (Kumar, 1986), membrane processes (Fuh & Chiang, 1990; Giovanetto, 1990; Shi, Kumar, & Kutowi, 2000) and supercritical fluids (Kienle, 1992).

Rank and Midmore (2006) classified the refining methods of stevioside into solvent partition extraction mainly methanol or water extraction and solvent partition extraction, incorporating mainly *in situ* precipitation with calcium hydroxide–carbon dioxide to remove impurities, similar to the purification process in the sugar industry. They also reported different methods of purification, such as adsorption, chromatography, ion-exchange, plasmid gel or adsorption by activated carbon. Hot water appeared to be the preferred medium for extraction, since the better-tasting rebaudioside A was more soluble than stevioside in water. However, some patents claimed many advantages in the use of solvents, such as ethanol, methanol/chloroform, glycerin, sorbitol or propylene glycol. Liu, Ong, and Li (1997) extracted stevioside from dried leaves of *S. rebaudiana* with hot methanol. They also studied the extraction of steviol glycosides, like rebaudioside A, rebaudioside C, and dulcoside A by subcritical fluid extraction (Sub FE). A simple efficient Sub FE method was developed and more than an 88%

extraction efficiency was obtained by using methanol as a modifier.

Stevioside is usually determined in *S. rebaudiana* by hot water leaching or supercritical fluid extraction (SFE) followed by liquid-chromatographic analysis of the extract (Pól et al., 2007). SFE employing CO_2 as a medium for extraction is faster than the previous method. It benefits from the physico-chemical properties of supercritical CO_2, which possesses a higher diffusivity and lower viscosity than conventional liquid solvents. However, pure CO_2 does not have sufficient solvation power for polar stevioside and therefore a polar co-solvent must be added. Investigated co-solvents were methanol, water, ethanol and mixtures of these solvents (Abou-Arab et al., 2010; Choi et al., 2002; Pasquel, Meireles, Marques, & Petenate, 2000; Pól et al., 2007; Yoda, Marques, Petenate, & Meireles, 2003).

Several methods are known for determining the quantitative content of glycosides in plant material (e.g. gas chromatography or infrared spectroscopy). However, the simplest and most reliable method is HPLC, which has been used to determine the composition of *S. rebaudiana* growing in various geographical areas (Kovylyaeva et al., 2007). Among the multiple glycosides, several complex glucosides have been determined in plants and cereals using high-performance liquid chromatography (HPLC), mass spectrometry (MS), nuclear magnetic resonance (NMR) or gas chromatography (GC) (Bernal et al., 2011). The determination of stevioside, rebaudioside A and steviol was carefully pursued through different methods as indicated in the scientific literature, including enzymatic hydrolysis and chemical detection, GC, over-pressure TLC, densitometry, HPLC and capillary electrophoresis (Gardana et al., 2010). HPLC technology and a near infrared (NIR) spectroscopy model was established to directly measure the stevioside glycosides (rebaudioside A and stevioside) content in the leaves of *S. rebaudiana* Bertoni. This model can be applied directly to measure the content of rebaudioside A and stevioside in the leaves of *S. rebaudiana* Bertoni, and resolved the problem of high cost and complex operation in using the current chemical laboratory methods (Yu, Xu, & Shi, 2011).

A qualitative LC-TOF method was also proposed to evaluate steviol glycosides (Pól, Hohnová, et al., 2007) together with a validated HPTLC procedure with densitometric detection (Jaitak, Gupta, Kaul, & Ahuja, 2008) and a NIRS procedure for the quantification of steviol glycosides (Hearn & Subedi, 2009). Recently, a semi-quantitative determination of steviol glycosides was also performed by desorption electrospray ionization mass spectrometry (Jackson et al., 2009). As for steviol quantification, Minne, Compernolle, Topped, and Geuns (2004) validated an RP-LC method with fluorometric detection after derivatization by a coumarin by-product.

7. Antimicrobial activity

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and re-emerging infectious diseases and development of resistance to the antibiotics in current clinical use (Cowan, 1999). The screening of plant extracts has been of great interest to scientists in the search for new drugs for effective treatment of several diseases. Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Jayaraman et al., 2008). The results of an investigation performed in the late 19th and 20th century and the advent of streptomycin and other antibiotics provide the ground for experimentation of a vast number of plants for antibiotic or antimicrobial activities that are useful to man (Doss & Dhanabalan, 2009).

Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds. In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids. Plants have an almost limitless ability to synthetize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. These compounds protect the plant from microbial infection and deterioration. Some of these phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects. Some preclinical studies suggest that phytochemicals can prevent colorectal cancer and other cancers (Jayaraman et al., 2008).

Stevia is thought to inhibit the growth of certain bacteria and other infectious organisms (Patil et al., 1996; Sivaram & Mukundam, 2003). Some people even claim that using Stevia helps to prevent the onset of colds and flu. The ability of Stevia to inhibit growth of certain bacteria 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 (Debnath, 2008).

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many researchers. In some studies the antimicrobial activity of various extracts of *S. rebaudiana* (with water, acetone, chloroform, methanol, ethyl acetate or hexane as solvents) have been investigated and its effect on some selected microorganisms such as *Salmonella typhi, Aeromonas hydrophila, Vibrio cholerae, Bacillus subtilis, Staphylococcus aureus* and others have been examined (Debnath, 2008; Ghosh, Subudhi, & Nayak, 2008; Jayaraman et al., 2008; Seema, 2010; Tadhani & Subhash, 2006b). The biological activity for *Stevia* compounds has been studied by Tomita et al. (1997); they studied the bactericidal activity of a fermented hot-water extract from *S. rebaudiana* Bertoni towards enterohaemorrhagic *Escherichia coli* and other food-borne pathogenic bacteria. Other microorganisms like *Salmonella typhimurium, B. subtilis*, and *S. aureus* has also been found to be inhibited by the fermented leaf extract (Debnath, 2008; Ghosh et al., 2008).

8. Antioxidant activity

Antioxidants are compounds that have gained importance in recent years due to their ability to neutralize free radicals (Devasagayam et al., 2004). Antioxidants have been reported to prevent oxidative damage caused by free radicals. They can interfere with the oxidation process by reacting with the free radicals, chelating catalytic metals and also acting as oxygen scavengers (Buyukokuroglu, Gulcin, Oktay, & Kufrevioglu, 2001). The antioxidant compounds present in edible plants have recently been promoted as food additives since they display little or no toxic side effects (Seong, Seog, Yong, Jin, & Seung, 2004). Many of the biologically active substances found in plants, including phenolic compounds (flavonoids, phenolics) are known to possess potential antioxidant properties. The antioxidant activity of medicinal plants depends on the concentration of the individual antioxidant entering into the composition (Shukla, Mehta, Mehta, & Bajpai, 2011).

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, they are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Natural plant-based antioxidants, especially phenolics and flavonoids have been exploited commercially either as antioxidant additives or as nutritional supplements (Schuler, 1990). Many other plant species have also been investigated in the search for novel antioxidants (Chu, Chang, & Hsu, 2000). However there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant potential (Shukla et al., 2011).

There are many different antioxidants present in plants and it is very difficult to measure each antioxidant component separately. Therefore, several methods have been developed to evaluate the antioxidant activity of fruits or other plants and animal tissues. Among them, Trolox equivalent antioxidant capacity (TEAC), total radical absorption potentials (TRAP), oxygen radical absorption capacity (ORAC), as well as the ferric reducing ability of plasma (FRAP) are commonly used and are the representative methods frequently used in scientific investigations (Tadhani, Patel, & Subhash, 2007). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is another method that can accommodate a large number of samples in a short period of time and is sensitive enough to detect natural compounds at low concentrations (Ahmad, Fazal, Abbasi, & Farooq, 2010), where the antioxidant activity is determined as the percentage inhibition of the DPPH free radical (Turkmen, Sari, & Velioglu, 2005). The DPPH method is widely reported for the screening of antioxidants and for determining comparative antioxidant effectiveness (Vani, Rajani, Sarkar, & Shishoo, 1997).

Some authors have reported values of the antioxidant capacity of *Stevia* (Table 7), determined in terms of percent inhibition of DPPH radicals and IC_{50} (concentration required for 50% inhibition of DPPH radicals), where a higher DPPH radical scavenging activity is associated with a lower IC_{50} value.

Phansawan and Poungbangpho (2007) studied the antioxidant capacities of five different medicinal plants; *Pueraria mirifica*, *S. rebaudiana* Bertoni, *Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) Nees. and *Cassia alata* Linn. The method was based on inhibition in absorption of ABTS (2,2′-azinobis(3-ethylbenzothiazolline-6-sulphonic acid) technique and the antioxidant capacity was recorded as TEAC. The medicinal plants were subjected to extraction with five solvents including ethanol, methanol, acetone, acetic acid, and distilled water, where the highest antioxidant capacity was found in *S. rebaudiana* Bertoni, followed by *C. alata* Linn. and *C. longa* Linn. *A. paniculata* (Burm.f.) Nees. and *P. mirifica* had the lowest antioxidant capacity. The highest antioxidant capacity was found in *S. rebaudiana* Bertoni extracted with acetone and methanol, followed by *S. rebaudiana* Bertoni extracted with ethanol, and *C. alata* Linn. extracted with ethanol, and the lowest antioxidant capacity was found in *Andrographis paniculata* (Burm.f.) Nees. extracted with acetone.

Stevia leaf extract exhibits a high degree of antioxidant activity and has been reported to inhibit hydroperoxide formation in sardine oil with a potency greater than that of either DL-α-tocopherol or green tea extract. The antioxidant activity of *Stevia* leaf extract has been attributed to the scavenging of free radical electrons and superoxides (Thomas & Glade, 2010). A recent study assessing the *in vitro* potential of ethanolic leaf extract of *S. rebaudiana* indicates that it has a significant potential for use as a natural antioxidant (Shukla, Mehta, Bajpai, & Shukla, 2009).

Table 7
DPPH radical scavenging activities of different leaf and callus extract of *Stevia rebaudiana* B.

Stevia rebaudiana B.	Inhibition Percentage	IC_{50} ($\mu g\ ml^{-1}$)	Extract	References
Leaf	64.26 [a]	83.45	Aqueous	Shukla et al. (2011)
Leaf	62.76 [a]	93.46	Ethanolic	Shukla et al. (2009)
Leaf	39.86 [b]	752.6	Aqueous	Tadhani et al. (2007)
Callus	55.42 [b]	541.3	Aqueous	Tadhani et al. (2007)
Leaf	33.17 [b]	904.4	Methanolic	Tadhani et al. (2007)
Callus	56.82 [b]	527.9	Methanolic	Tadhani et al. (2007)
Leaf	77.67 [c]	ND	Methanolic	Ahmad et al. (2010)
Leaf	67.08 [c]	ND	Ethanolic	Ahmad et al. (2010)
Leaf	ND	45.32	Aqueous	Ghanta et al. (2007)
Leaf	ND	47.66	Methanolic	Ghanta et al. (2007)
Leaf	82.86	5.00	Aqueous	Muanda, Soulimani, Diop, and Dicko (2010)
Leaf	96.91	2.90	Methanolic/aqueous	Muanda et al. (2010)
Leaf	10.15 [a]	ND	Aqueous	Kim et al. (2011)
Callus	3.50 [a]	ND	Aqueous	Kim et al. (2011)

Concentration of:
[a] 100 $\mu g\ ml^{-1}$.
[b] 600 $\mu g\ ml^{-1}$.

9. Health benefits

Many plant glycosides have shown activity in cancer prevention, as well as antidiabetic, anti-obesity, antibacterial or antineoplastic effect (Bernal et al., 2011). *S. rebaudiana* leaves contain non-cariogenic and non-caloric sweeteners (steviol glycosides) whose consumption could exert beneficial effects on human health (Gardana et al., 2010). Stevia glycosides possess valuable biological properties. Regular consumption of these compounds decreases the content of sugar, radionuclides, and cholesterol in the blood (Atteh et al., 2008), improves cell regeneration and blood coagulation, suppresses neoplastic growth and strengthens blood vessels (Barriocanal et al., 2008; Jeppesen et al., 2003; Maki et al., 2008; Wingard et al., 1980). They also exhibit choleretic (Kochikyan et al., 2006), anti-inflammatory (Jayaraman et al., 2008; Sehar, Kaul, Bani, Pal, & Saxena, 2008) and diuretic properties; they prevent ulceration in the gastrointestinal tract (Kochikyan et al., 2006), including antihypertensive (Chan et al., 2000; Jeppesen, Gregersen, Gregersen, Alstrupp, & Hermansen, 2002; Lee, Wong, Liu, Chen, & Chan, 2001), antihyperglycemic (Chen et al., 2006; Jeppesen, Gregersen, Poulsen, & Hermansen, 2000; Jeppesen et al., 2002; Suanarunsawat & Chaiyabutr, 1997); anti human rota-virus activities (Suanarunsawat & Chaiyabutr, 1997; Takahashi et al., 2001), glucose metabolism (Suanarunsawat & Chaiyabutr, 1997; Toskulkao, Sutheerawatananon, Wanichanon, Saitongdee, & Suttagit, 1995) and renal function (Jutabha, Toskulkao, & Chatsudthipong, 2000). They present potential applications as antidiarrhoeal therapeutics (Chatsudthipong & Muanprasat, 2009). In addition, the *Stevia* plant and stevioside have been used in the treatment of cancer and as substitutes for saccharose in the treatment of diabetes (Chen et al., 2006; Jeppesen et al., 2000; Pól, Hohnová, et al., 2007), obesity and hypertension (Chan et al., 2000; Goyal et al., 2010; Hsieh et al., 2003; Lee et al., 2001; Pól, Hohnová, et al., 2007). They can also act as an anti-cariogenic product (Blauth de Slavutzky, 2010; Das et al., 1992; Suanarunsawat & Chaiyabutr, 1997), and as antigingivitis (Blauth de Slavutzky, 2010).

The toxicology of stevioside has been extensively studied, and related data, reassessed lately, indicated it to be non-toxic, non-mutagenic, and non-carcinogenic. It was also clearly demonstrated that high concentrations of the sweetener rebaudioside A, administered in the diet of rats over 90 days, were not associated with any signs of toxicity (Gardana et al., 2010) and no allergic reaction have been observed when it is used as a sweetener (Abou-Arab et al., 2010). A number of studies have demonstrated that oral intake of stevioside has no effect on fertility, neither in mice (Akashi & Yokoyama, 1975), nor in rats (Mori, Sakanoue, Takcuchi, Shimpo, & Tanabe, 1981; Xili et al., 1992), nor in hamsters (Yodyingyuad & Bunyawong, 1991). Adverse effects of stevia have not really been observed. Its commercialisation, in France for example, as a food or a food ingredient has been prohibited based mainly on economical arguments and not on proven adverse health effects (Serio, 2010). However, it is thought that stevia could provoke allergic reactions in people sensitive to plants of the Asteraceae family and it is also recommended that pregnant women should avoid consuming stevia (Serio, 2010).

10. Industrial applications

Stevia sweeteners, extracts from the leaves of this herb, are commercially available in Japan, Korea, China, South-East Asia and South America, where they have been used for some decades to sweeten a variety of foods (Koyama et al., 2003). In these countries stevioside is being used to sweeten foodstuffs and beverages. In the USA powdered *Stevia* leaves and their extracts are used only as a dietary supplement and a skin care product, but not as a sweetener. Since December 2008 when the FDA stated that purified rebaudioside A (rebiana) from *Stevia* can be considered GRAS (Generally Recognised As Safe), rebiana has been in use to sweeten beverages and some foods (FDA GRAS Notice GRN 000253 and GRN 000252). In France too, as of 26 August 2009, purified rebiana (97%) has been authorised on a trial period of two years for use at a maximal permissible concentration in certain foodstuffs (Serio, 2010). On the other hand, steviol glycosides have not been approved by the European Commission arguing safety concern. However, in 2008 JECFA suggested a temporary admissible daily intake (ADI) of 0–4 mg kg^{-1} BW of steviol glycoside, an equivalent of 0–10 mg kg^{-1} BW of stevioside (Gardana et al., 2010). If not consumed in excess, steviol glycosides may be considered safe. As estimated by Serio (2010) a daily consumption of 400 mg steviol glycosides would produce through decomposition by bacteria in the large intestine only a negligible amount of glucose, about 80 mg, that will be resorbed.

The use of *S. rebaudiana* as a sweetener can be found in many parts of Central and South America, where this species is indigenous, as well as in Japan (Goyal et al., 2010). The leaves of *Stevia* naturally contain a complex mixture of eight sweet diterpene glycosides, including stevioside, steviolbioside, rebaudiosides (A, B, C, D, E) and dulcoside A (Abou-Arab et al., 2010).The steviol glycosides are currently in use as a sweetener in a number of industrial foods, such as soft drinks or fruit drinks (Goyal et al., 2010; Jayaraman et al., 2008; Tadhani & Subhash, 2006a; Wallin, 2007), desserts, cold confectionery, sauces, delicacies, sweet corn, breads, biscuits, table-top sweetener. They replace saccharose, for example in ready-to-eat cereals (Wallin, 2007), pickles (Koyama et al., 2003), yoghurt (Amzad-Hossain et al., 2010; Tadhani & Subhash, 2006a; Wallin, 2007), candies (Goyal et al., 2010; Koyama et al., 2003), soju, soy sauce (Amzad-Hossain et al., 2010; Tadhani & Subhash, 2006a) and seafoods (Goyal et al., 2010; Koyama et al., 2003).

11. Conclusion

S. rebaudiana Bertoni is an ancient South American plant with great potential as an agricultural crop for the production of a high-potency natural sweetener. Owing to its proximate composition and its content of health-promoting phytochemical constituents, it is also a suitable raw material for the extraction and production of functional food ingredients. It is a good source of carbohydrates, protein, crude fibre, minerals, as well as dispensable and indispensable amino acids which are valuable for human nutrition. The sweetening compounds, found mainly in the leaves of the plant, are steviol glycosides, with stevioside being the most abundant, followed by rebaudioside A. Stevioside has a sweetening power comparable to that of artificial sweeteners presently marketed and consumed in several foods and beverages. It is about 300 times sweeter than saccharose. Rebaudioside A is known to be even sweeter (up to 450 times sweeter than saccharose) and can be refined to a purity of over 97%. The leaves, as well as the pure stevioside extract, can be used in its natural state or cooked, and are thermostable at temperature up to 200 °C. They are non-fermentative low-calorie, non-toxic sweeteners, flavour enhancing and have been tested objectively, based on direct observations on human and animals, showing them to be non-mutagenic, non-teratogenic and non-carcinogenic. Stevia has been consumed by human beings for centuries without any negative effects. This showed the advantages of stevia over other artificial sweeteners as an ingredient for the food industry, thereby making Stevia a more suitable substitute for saccharose in different drinks, beverages and bakery products. Apart from the sweet contents, *S. rebaudiana* with its secondary plant constituents also offers therapeutic benefits, having anti-hyperglycaemic, anti-hypertensive, anti-inflammatory, antitumour, anti-diarrhoeal, diuretic, and immunomodulatory effects.

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