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. © 2011 Elsevier Ltd. All rights reserved.

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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, Mianur-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, & Fodick, 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, Samshir, & Goyal, 2010). For centuries, the Guarani tribes of Paraguay and Brazil used Stevia species, primarily S. rebaudiana, which they called ka’ a he’è (“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 (Pöl, 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).

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 (Pöl, 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 steviosyl 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 perennia l 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 pentameric, 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

Fig. 1. Stevia rebaudiana Bertoni leaves.
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 Anambar 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 steviolose 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, Yamshi, 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 escalate 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. Cramer 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
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 caramelize 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 and anti-inflammatory activities (Bernal, Mendiola, Ibáñez, & Cifuente, 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 1

Proximate analysis of dried Stevia leaves (g 100 g⁻¹ dry weight basis).

<table>
<thead>
<tr>
<th>Component</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>Mishra et al. (2010)</td>
</tr>
<tr>
<td>Protein</td>
<td>Goyal et al. (2010)</td>
</tr>
<tr>
<td>Fat</td>
<td>Serio (2010)</td>
</tr>
<tr>
<td>Ash</td>
<td>Savita et al. (2004)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Abou-Arab et al. (2010)</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>Tadhani and Subhash (2006a)</td>
</tr>
</tbody>
</table>

ND, not determined.

### Table 2

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

<table>
<thead>
<tr>
<th>Essential amino acid g 100 g⁻¹ d.m.</th>
<th>Non-essential amino acid g 100 g⁻¹ d.m.</th>
<th>Report of a Joint WHO/FAO/UNU Expert Consultation (WHO, 2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine⁴</td>
<td>Aspartate</td>
<td>0.45</td>
</tr>
<tr>
<td>Lysine</td>
<td>Serine</td>
<td>0.70</td>
</tr>
<tr>
<td>Histidine</td>
<td>Glutamic</td>
<td>1.13</td>
</tr>
<tr>
<td>Phenyl alanine</td>
<td>Proline</td>
<td>0.77</td>
</tr>
<tr>
<td>Leucine</td>
<td>Glycine</td>
<td>0.98</td>
</tr>
<tr>
<td>Methionine</td>
<td>Alanine</td>
<td>1.45</td>
</tr>
<tr>
<td>Valine</td>
<td>Cysteine⁵</td>
<td>0.64</td>
</tr>
<tr>
<td>Threonine</td>
<td>Tyrosine⁶</td>
<td>1.13</td>
</tr>
<tr>
<td>Isoleucine</td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7.67</td>
</tr>
</tbody>
</table>


⁵ Cysteine included under specific situation.
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 synthesize 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.

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 3

<table>
<thead>
<tr>
<th>Minerals</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Mishra et al. (2010)</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>11.4</td>
</tr>
<tr>
<td>Sodium</td>
<td>190</td>
</tr>
<tr>
<td>Potassium</td>
<td>1800</td>
</tr>
<tr>
<td>Iron</td>
<td>55.3</td>
</tr>
<tr>
<td>Magnesium</td>
<td>349</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>g 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C16)</td>
<td>27.51</td>
</tr>
<tr>
<td>Palmitoleic acid (C16-1)</td>
<td>1.27</td>
</tr>
<tr>
<td>Stearic acid (C18)</td>
<td>1.18</td>
</tr>
<tr>
<td>Oleic acid (C18-1)</td>
<td>4.36</td>
</tr>
<tr>
<td>Linoleic acid (C18-2)</td>
<td>12.40</td>
</tr>
<tr>
<td>Linolenic acid (C18-3)</td>
<td>21.59</td>
</tr>
</tbody>
</table>
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 compounds are alkaloids, tannins and polyphenols (Halliwell, Gutteridge, & Arurma, 1987). It has been reported that the levels of plasma antioxidants 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.

### Table 5

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Leaf (mg 100 g⁻¹ dry base of extract)</th>
<th>Callus (mg 100 g⁻¹ dry base of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>14.98 ± 0.07</td>
<td>1.64 ± 0.02</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>0.43 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Folic acid</td>
<td>52.18 ± 0.21</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

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 foodstuff 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, & Shivandan, 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, steribins 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 (C₃₈H₄₆O₁₈) 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 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 possesses 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 (Kovylyeva and...
The sweetness of any of the stevia compounds is greater than that of saccharose: rebaudioside A (250–450 times); rebaudioside E (150–300 times); dulcoside A (100–125 times). The edulcorant properties of those glycosides, however, have also been studied in seeds, plants and cereals. Saponins can stimulate muscle growth and raise testosterone levels and they 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, & Iwama, 1978), chromatographic adsorption (Ahmed & Dobberstein, 1982; Kolb, Herrera, Ferreyra, & Uliana, 2001; Makapugay et al., 1984; Striedner, Czygan, & Braunegg, 1991), ion exchange (Fuh & Chiang, 1990; Giovannetto, 1990; Payzant, Laidler, & Ippolito, 1999) selective precipitation (Kumar, 1986), membrane processes (Fuh & Chiang, 1990; Giovannetto, 1990; Shi, Kumar, & Kutow, 2000) and supercritical fluids (Kiene, 1992).

Rank and Midmore (2006) classified the refining methods of stevioside to 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%
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 synthesize 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).

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).
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, & Veligolu, 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.

### Table 7

<table>
<thead>
<tr>
<th>Stevia rebaudiana B.</th>
<th>Inhibition Percentage</th>
<th>IC_{50} (μg ml^{-1})</th>
<th>Extract</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>64.26 (^{a})</td>
<td>83.45</td>
<td>Aqueous</td>
<td>Shukla et al. (2011)</td>
</tr>
<tr>
<td>Leaf</td>
<td>62.76 (^{a})</td>
<td>91.46</td>
<td>Ethanolic</td>
<td>Shukla et al. (2009)</td>
</tr>
<tr>
<td>Leaf</td>
<td>39.86 (^{b})</td>
<td>752.6</td>
<td>Aqueous</td>
<td>Tadhani et al. (2007)</td>
</tr>
<tr>
<td>Calssus</td>
<td>55.42 (^{b})</td>
<td>541.3</td>
<td>Aqueous</td>
<td>Tadhani et al. (2007)</td>
</tr>
<tr>
<td>Leaf</td>
<td>33.17 (^{b})</td>
<td>904.4</td>
<td>Methanolic</td>
<td>Tadhani et al. (2007)</td>
</tr>
<tr>
<td>Calssus</td>
<td>56.82 (^{b})</td>
<td>527.9</td>
<td>Methanolic</td>
<td>Tadhani et al. (2007)</td>
</tr>
<tr>
<td>Leaf</td>
<td>77.67 (^{b})</td>
<td>ND</td>
<td>Methanolic</td>
<td>Ahmad et al. (2010)</td>
</tr>
<tr>
<td>Leaf</td>
<td>67.08 (^{c})</td>
<td>ND</td>
<td>Ethanolic</td>
<td>Ahmad et al. (2010)</td>
</tr>
<tr>
<td>Leaf</td>
<td>ND</td>
<td>45.32</td>
<td>Methanolic</td>
<td>Ghanta et al. (2007)</td>
</tr>
<tr>
<td>Leaf</td>
<td>ND</td>
<td>47.66</td>
<td>Methanolic</td>
<td>Ghanta et al. (2007)</td>
</tr>
<tr>
<td>Leaf</td>
<td>82.86</td>
<td>5.00</td>
<td>Aqueous</td>
<td>Muanda, Soulimani, Diop, and Dicko (2010)</td>
</tr>
<tr>
<td>Leaf</td>
<td>96.91</td>
<td>2.90</td>
<td>Methanolic/ aqueous</td>
<td>Muanda et al. (2010)</td>
</tr>
<tr>
<td>Callus</td>
<td>10.15 (^{a})</td>
<td>ND</td>
<td>Aqueous</td>
<td>Kim et al. (2011)</td>
</tr>
<tr>
<td>Callus</td>
<td>3.30 (^{a})</td>
<td>ND</td>
<td>Aqueous</td>
<td>Kim et al. (2011)</td>
</tr>
</tbody>
</table>

Concentration of:

- \(^{a}\) 100 μg ml^{-1}
- \(^{b}\) 600 μg 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 (stevial 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, Gregerson, Gregerson, Alstrupp, & Hermansen, 2002; Lee, Wong, Liu, Chen, & Chan, 2001), antihyperglycemic (Chan et al., 2006; Jeppesen, Gregerson, Poulsen, & Hermansen, 2000; Jeppesen et al., 2002; Suanunrasawat & Chaiyabutr, 1997), anti human rota-virus activities (Suanunrasawat & Chaiyabutr, 1997; Takahashi et al., 2001), glucose metabolism (Suanunrasawat & Chaiyabutr, 1997; Toskulkao, Sutheerawatananon, Wanichanon, Saitongdee, & Suttagit, 1995) and renal function (Jutabha, Toskulkao, & Chatsudthipong, 2000). They present potential applications as anti-diarrhoeal and C. longa Linn. extracted with ethanol, and *A. paniculata* (Burm.f.) Nees. 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.

*S. rebaudiana* leaf extract exhibits a high degree of antioxidant activity and has been reported to inhibit hydroperoxide formation in sar-dine oil with a potency greater 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 & Glaude, 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).
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 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 stevioside 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 stevioside 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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