Stevia Rebaudiana Bertoni (Honey Leaf): A Magnificent Natural Bio-sweetener, Biochemical Composition, Nutritional and Therapeutic Values

Abdelkarim Khiraoui[1,2*] Aziz Hasib[1] Chaouki Al Faiz[2] Fatimazahra Amchra[2] Mohamed Bakha[2] Abdelali Boulli[1]
1.Laboratory of Environment and Valorisation of Agro resources, University Sultan Moulay Slimane. Faculty of Sciences and Technology, BP: 523, Mhgila Beni Mellal, Morocco
2.National Institute of Agricultural Research, UR Aromatic and Medicinal Plants INRA, CRRA-Rabat, PB 6570, 10101 Rabat, Morocco

Abstract
Stevia rebaudiana Bertoni (Bert.) is a nutrient rich natural alternative to artificial sweetener, belongs to Asteraceae family and contains over hundred phytochemicals. The most important components of *S. rebaudiana* is steviol glycosides, and that thanks to its high potency sweetening which estimated about 300 times sweeter than saccharose but besides this has no calorific value. Many research activities on its biochemical and biological properties have been done in recent past. Accordingly, stevia has numerous therapeutic values in the treatment of patients with diabet-related obesity, hypertension or cardiac disease, antioxidant, antimicrobial and antifungal activity, for which sweetening properties have been identified. The aim of this review is to present biochemical composition, nutritional value and application of stevia leaves and its beneficial value to health.
Keywords: *Stevia rebaudiana*, Asteraceae, biochemical, biological properties, sweetener, nutritional.

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 et al., 2010). Among the 230 species in the genus *Stevia*, only the species *rebaudiana* and *phlebophylla* produce steviol glycosides (Brandle et Telmer, 2007).

Stevia rebaudiana (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 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).

Figure 1. *Stevia rebaudiana* Bertoni cultivated in Morocco

S. rebaudiana 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 et al., 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, antihypertensive, 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 (Pól et al., 2007).

The purpose of this review is to selection of essential information coming from several scientific researches on stevia, a naturally bio-sweetener. importance was placed on the amazing potential of stevia as an intense high-potency sweetener with its nutritional, therapeutic, functional properties and health-promoting properties.

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 et al., 2010), 60 cm to 1m tall (Serio, 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 (Lemus-Modaca et al., 2012).

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 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 (Lemus-Modaca et al., 2012). 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.

3. Biochemical and nutritional aspects of stevia

The dry extract from the leaves of stevia contains flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycinnamic acids (caffeine, chlorogenic, etc.), neutral water-soluble oligosaccharides, free sugars, amino acids, lipids, essential oils and trace elements (Komissarenko et al., 1994).

Savita et al., 2004 analysed stevia leaves on a dry weight basis and calculated an energy value of 2.7 kcal g^{-1}, moreover, Khiraoui et al., 2017 being analyzed were found to be (3.05 - 3.17 kcal g^{-1}) This means that stevia may be granted the status of a low calorie sweetener. 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 (Lemus-Modaca et al., 2012).

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.

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 the preparation of viscous foods such as soups, gravies, dough and baked products. The ability of the protein to aid the formation and stabilization of emulsion is also critical in many foods applications, such as cake, batters, coffee whiteners, milk, frozen desserts and others. 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 flavor retainers and increases mouthfeel of foods.

The benefits associated with stevia leaf are principally due to their biochemical and nutritional composition (Table 1), which is a good source of carbohydrates, protein and crude fiber that promotes wellness and minimize the risk of certain diseases.

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

Components	References											
	Savita et al. (2004)	Tadhani & Subhash, (2006)	Mishra et al. (2010)	Goyal et al. (2010)	Serio (2010)	Kaushik et al. (2010)	Abou-Arab et al. (2010)	Atteh et al. (2011)	Woelwer-Rieck, 2012	Segura et al. (2014)	Gasmalla et al. (2014)	Khiraoui et al. (2017)
Moisture	7	ND	7	4.65	ND	7.7	5.37	ND	7.2-8.8	7.45-7.80	4.45-10.73	4.97-8.31
Protein	9.8	20.4	10	11.2	11.2	12	11.40	16	12.1- 15.5	12.11-15.05	12.44-13.68	11.75-16.23
Fat	2.5	4.34	3	1.9	5.6	2.7	3.73	2.6	3.6-5	3.04-3.23	4.18-6.13	3.86-5.78
Ash	10.5	13.1	11	6.3	ND	8.4	7.41	15.5	7.7-8.1	7.82-11.93	4.65-12.06	7.37-11.28
Carbohydrates	52	35.2	52	ND	53	ND	61.9	ND	ND	64.06-67.98	63.10-73.99	51.50-56.72
Crude fiber	18.5	ND	18	15.2	15	ND	15.5	6.8	9.7-12.1	5.92-9.52	4.35-5.26	17.43-19.13

ND, not determined.

3.1 Carbohydrates

Carbohydrates are the principal sources of energy and they are found as structural components of cellular elements (Lemus-Modaca et al., 2012). The advantages associated with stevia leaf are mainly due to their nutritional composition (Table 1), which is a good source of 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 et al., 2011). In *S. rebaudiana* roots and leaves, inulin-type fructo-oligosaccharides at 4.6%, a naturally occurring plant polysaccharide with important functional properties related to prebiotics, dietary fiber, role lipid metabolism and diabetes control, have been isolated by Braz de Oliveira et al. (2011).

3.2 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).

The study of Mohammad et al., (2007) identified eight essential amino acids in stevia leaves, namely glutamic acid, aspartic acid, lysine, serine, isoleucine, alanine, proline, tyrosine . Abou-Arab et al. (2010) and Li et al. (2011) had 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).

Table 2. Essential and non-essential amino acids content of stevia leaves (g 100 g^{-1} dry weight basis)

Amino acids	**References**	
Essential amino acid	Abou-Arab et al. (2010)	Li et al. (2011)
Arginine	0.45	0.81
Lysine	0.70	0.15
Histidine	1.13	0.34
Phenyl alanine	0.77	0.88
Leucine	0.98	1.30
Methinine	1.45	ND
Valine	0.64	0.94
Threonine	1.13	0.75
Isoleucine	0.42	0.72
Non-essential amino acid		
Aspartate	0.37	1.72
Serine	0.46	1.02
Glutamic	0.43	1.90
Proline	0.17	1.72
Glycine	0.25	0.85
Alanine	0.56	0.95
Cysteine	0.40	ND
Tyrosine	1.08	0.49

ND, not determined

3.3 Crude fiber

Dietary fiber is the comestible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Sankhala et al., 2005). Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. In addition, an analogous carbohydrate is defined as those carbohydrates-based food ingredients that are non-digestible and non-absorbable, and which are similar to plant dietary fiber (Prosky, 2001). Dietary fiber has been widely studied for its health benefits. It is considered a preventive factor for cancer, serves as a substrate for colonic bacteria, promotes intestinal food transit, and decreases bile acid reabsorption thereby altering micelle formation and contributing to lowering blood cholesterol levels (Escudero & González, 2006).

3.4 Minerals

Minerals have many important functions in the human body. 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 (Adoteyet al., 2009). 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 (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.

Table 3: Minerals content (mg 100 g^{-1}) of dried stevia leaves

Minerals	**References**								
	Savita et al. (2004)	Tadhani et Subhash (2006a)	Goyal et al. (2010)	Serio (2010)	Mishra et al. (2010)	Kaushik et al. (2010)	Abou-Arab et al. (2010)	Atteh et al. (2011)	Khiraoui et al. (2017)
Calcium	ND	1550	544	600	464.4	722	17.7	8.2	579.68-734,57
Phosphorus	11.4	350	318	318	11.4	ND	ND	2.6	ND
Sodium	ND	160	89.2	ND	190	32.7	14.93	0.7	69.87-190.14
Potassium	190	2510	1780	1800	1800	839	21.15	17.3	1421.24-2787.11
Iron	55.3	36.3	3.9	3.9	55.3	31.1	5.89	366	5.73-35.44
Magnesium	1800	ND	349	500	349	ND	3.26	2.4	179.57-198.18
Zinc	ND	6.39	1.5	ND	1.5	ND	1.26	20	1.71-5.32

ND, not determined

3.5 Lipids

Lipids are a large group of natural compounds. Their main biological functions include energy storage (Bernal et al., 2011). In the leaf oil of stevia, Tadhani and Subhash (2006a) and Atteh et al. (2011) had identified six fatty acids using methyl ester standards. Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were identified in the leaf oil (Table 4).

Table 4. The composition of fatty acids contents (g 100 g^{-1}) in oil from stevia leaves

Fatty acids	**References**	
	Tadhani & Subhash (2006)	Atteh et al. (2011)
Palmitic acid	27.51	29.5
Oleopalmitic acid	1.27	3.0
Stearic acid	1.18	4.0
Oleic acid	4.36	9.9
Linolic acid	12.40	16.8
Linolenic acid	21.59	32.6

3.6 Vitamins

Kim et al. (2011) studied the amounts of water-soluble vitamins in the stevia leaf 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. Water soluble vitamins of *S. rebaudiana* leaf (mg 100 g^{-1} dry basis of extract)

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 ± 0.02
Niacin	0.00 ± 0.00	0.00 ± 0.00
Thiamin	0.00 ± 0.00	0.00 ± 0.00

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 (Edeoga et al., 2005).The phytochemicals present in *S. rebaudiana* are austroinullin, β-carotene, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol, stevioside and thiamine (Jayaraman et al., 2008).

4.1. Diterpene glycosides

Glycosides are a group of organic 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) (Bernal et al., 2011).

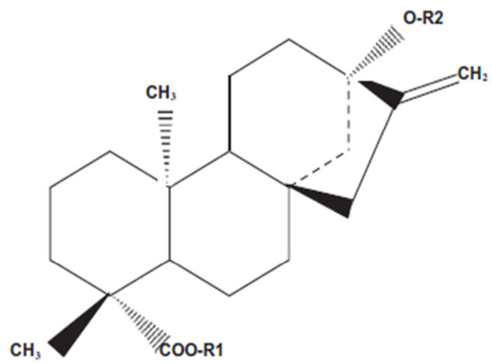
Stevia, the common name for the extract stevioside from the leaves of *S. rebaudiana*, is a new promising renewable raw food stuff on the world market and is a natural, sweet tasting calorie free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners (Anton et al., 2010). 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). Depending on growth conditions, cultivation and tillage techniques their contents range from 4 to 20% fresh leaf weight (Pól et al., 2007). 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 et al, 1984). Steviolbioside, rebaudioside B, D, E and F were also identified in the leaf extracts, but as minor constituents (Geuns, 2003). According to Mishra et al. (2010) the stevioside is the main sweetening compound found in the leaf of plant *Stevia rebaudiana* (from 5-15% dry weight), followed by rebaudioside (3-6%). The stevioside and the other stevia glycosides have high chemical stability because of their tridimensional chemical form which produces resistance to acid and enzymatic hydrolysis ensuring their inalterability even under biochemical and physiological aspects. Stevia are shown in the Table 6.

Table 6. Amount of sweet glycosides in stevia leaves

Steviol glycosides	**Contents, % of the leaves dry weight**											
	Kinghorn & Soejarto (1985)	Crammer & Ikan (1987)	Kolb et al. (2001)	Kovylyaeva et al. (2007)	Lavini et al. (2008)	Gardana et al. (2010)	Goyal et al. (2010)	Kaushik et al. (2010)	Serio (2010)	Atteh et al. (2011)	Jaworska et al. (2012)	Khiraoui et al. (2017)
Stevioside	5-10	3-10	3.78-9.75	4.8-5.8	5.72	5.8±1.3	9.1	11.3	6-7	6.5	2	6.26-10.10
Rebaudioside A	2-4	1	1.62-7.27	1.2-1.3	8.36	1.8±1.2	3.8	ND	1-4	2.3	5	ND
Rebaudioside C	1-2	ND	ND	0.3-0.5	ND	1.3±1.4	0.6	ND	1-2	ND	2	ND
Dulcoside A	0.4-0.7	0,2	ND	ND	ND	ND	0.3	ND	0.2-0.7	ND	1	ND

ND, not determined

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 et al., 2006).



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) |β-Glc(3→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) |β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) |β-Glc(3→1)
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) |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) |β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)

Figure 2. Structure of the major glycosides of *Stevia rebaudiana* leaves. Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

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 et 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 (Gardana et al., 2010). And compared stevia leaf powder and stevia white extract with granulated sugar (Table 7).

Table 7. Comparison of stevia leaf powder and stevia white extract with granulated sugar (Goyal et al., 2010)

Granu lated sugar	Stevia leaf powder	Stevia white extract
1 teaspoon	1/8 teaspoon	Dust on spoon
1 tablespoon	3/8 teaspoon	1/2 pinch
1/4 cup	1/2 teaspoon	Pinch
1/2 cup	1 tablespoon	1/8 teaspoon
1 cup	2 tablespoons	1/4 teaspoon
3.75 pounds	7.2 ounces	0.3 ounces
10 pounds	19.2 ounces	0.8 ounces

5. Antioxidants activity

Active oxygen free radicals have been implicated as causative agents of cancer, atherosclerosis, cerebral and cardiac ischemia, Parkinson's disease, gastrointestinal disturbances and aging (Ames et al., 1993). Many herbal and some common medicinal plants are good sources of antioxidant compounds, including phenolic compounds (flavonoid, phenolics) are known to possess potential antioxidant properties (Larson, 1988). Moreover, stevia leaves have a high amount of phenolic compounds, vitamin C, carotenoids, chlorophylls (Abou-Arab et al., 2010).

Stevia leaf extract exhibits a high degree of antioxidant activity. 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 et al., 2009). Leaves of *S. rebaudiana* were found to contain polyphenolic compounds exhibiting antioxidant properties (Table 8 & 9)

Table 8. DPPH radical scavenging activities of different leaf extract of *S. rebaudiana*

Stevia rebaudiana	**Inhibition Percentage**	**IC50 (µg ml^{-1})**	**Extract**	**References**
Leaf	39.86 [b]	752.6	Aqueous	Tadhani et al. (2007)
Leaf	33.17 [b]	904.4	Methanolic	Tadhani et al. (2007)
Leaf	ND	45.32	Aqueous	Ghanta et al. (2007)
Leaf	ND	47.66	Methanolic	Ghanta et al. (2007)
Leaf	62.76 [a]	93.46	Ethanolic	Shukla et al. (2009)
Leaf	67.08	ND	Ethanolic	Ahmad et al. (2010)
Leaf	77.67	ND	Methanolic	Ahmad et al. (2010)
Leaf (oil)	82.86	5.00	Aqueous	Muanda et al. (2011)
Leaf	96.91	2.90	Methanolic/ aqueous	Muanda et al. (2011)
leaf	10.15 [a]	ND	Aqueous	Kim et al. (2011)
Leaf	64.26 [a]	83.45	Aqueous	Shukla et al. (2012)

ND, not determined
Concentration of:
[a] 100 µg ml^{-1}.
[b] 600 µg ml^{-1}.

Table 9: Total phenolic and flavonoid content (mg/g) of different leaf extract of *S. rebaudiana*

Stevia rebaudiana	**Total phenolic**	**flavonoid**	**Extract**	**Reference**
leaf	25.25	23.46	Methanolic	Liu et al. (2003)
leaf	25.73	21.73	Methanolic	Tadhani et al. (2007)
leaf	61,50	ND	Ethanolic	Shukla et al. (2009)
leaf	61.5	ND	Ethanolic	Serio, (2010)
leaf	130.67	15.64	aqueous	Kim et al. (2011)
leaf	56.73	ND	aqueous	Shukla et al. (2012)
leaf	55.64-58.35	48.29-60.33	Ethanolic	Zeng et al. (2013)

ND, not determined

6. 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).

Savita et al. (2004) found a high percentage of anti-nutritional factors in extracts of stevia leaf dissolved in water: oxalic acid and tannins. 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).

7. Principle Advantages of stevia

The principle advantages of stevia cultivation are given below:

- ✓ stevia is a completely natural non-synthetic product: Stevioside (the sweetener) contains absolutely no calories;
- ✓ The leaves can be used in their natural state;
- ✓ It has enormous sweetening power; only small quantities need to be used;
- ✓ The plant is non-toxic;
- ✓ The leaves as well as the pure stevioside extract can be cooked;
- ✓ Stable when heated up to 200°C;
- ✓ Non-fermentative;
- ✓ Flavour enhancing;
- ✓ Clinically tested and frequently used by humans without negative effect (Mishra et al., 2010).

8. 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).As toxicological studies has shown, that stevioside does not have mutagenic, teratogenic or carcinogenic effects and no allergic reaction have been observed when it is used as a sweetener. Stevia and stevioside have been applied as substitutes for sucrose, for treatment of diabetes mellitus, obesity, hypertension, and for the prevention of caries. Stevioside also exhibits bactericidal activity and inhibits the growth of Escherichia coli. (Pól et al., 2007). They can also act as an anti-cariogenic product, anti-gingivitis and be a helper in obesity because it is a sweetener that is not metabolized, that is, it has no calories, and therefore, it is not fattening (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).

9. Stevia as Functional Component for Food industry

Stevia rebaudiana is a natural sweet herb native; the Sweetness equivalence of stevia to sugar was carried out for threshold test. Sweetness of 1 g of stevia in 100 ml water was equivalent to a sucrose solution containing 20 g of sucrose. Similar results were found by (Cardello et al, 1999).

The use of stevia as a low calorie sweetener could be of immense help in restricting the calorie intake in the diet of affluent and also where in calorie restricted diet are prescribed (Mishra 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 ice creams, cookies, pickles, chewing gum, tea, candies, seafood's and skincare products (Goyal et al., 2010), desserts, cold confectionery, sauces, delicacies, sweet corn, breads, biscuits, table-top sweetener. They replace saccharose, for example in ready-to-eat cereals, yoghurt (Wallin, 2007), soju, soy sauce (Amzad-Hossain et al., 2010).

10. Uses of stevia

Stevia is safe for diabetics, as it does not affect blood sugar levels, not have the neurological or renal side effects as other artificial sweeteners and also, Stevia possess anti-fungal and anti-bacterial properties in addition to its other versatile uses; It can be safely used in herbal medicines, tonics for diabetic patients and in daily usage products such as mouthwashes and toothpastes, moreover mild stevia leaf tea offers excellent relief for an upset stomach (Goyal et al., 2010).

Conclusion

Stevia represents a new opportunity for researchers and farmers alike. A great deal of information relating to production practices and disease control is required to optimize annual production. The production of remarkably high levels of one class of secondary metabolite is of significant interest to chemists, biochemists and geneticists and may prove to be a foundation for the production of new metabolites in the future.

References

Abou-Arab, A., Abou-Arab, A., et Abu-Salem, M. F. (2010). Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* Bertoni plant. *African Journal of Food Science*, 4, 269–281.

Adotey, D., Serfor-Armah, Y., Fianko, J., & Yeboah, P. (2009). Essential elements content in core vegetables grown and consumed in Ghana by instrumental neutron activation analysis. *African Journal of Food Science*, 3, 243–249.

Ahmad, N., Fazal, H., Abbasi, B. H., & Farooq, S. (2010). Efficient free radical scavenging activity of Ginkgo biloba, *Stevia rebaudiana* and Parthenium hysterophorous leaves through DPPH (2, 2-diphenyl-1-picrylhydrazyl). *International Journal of Phytomedicine*, 2(3).

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences*, 90(17), 7915-7922.

Amzad-Hossain, M., Siddique, A., Mizanur-Rahman, S., et Amzad-Hossain, M. (2010). Chemical composition of the essential oils of *Stevia rebaudiana* Bertoni leaves. *Asian Journal of Traditional Medicines*, 5, 56–61.

Anton, S., Martin, C., Han, H., Coulon, S., Cefalu, W., Geiselman, P., et al. (2010). Effects of Stevia, aspartame, and sucrose on food intake, satiety and postprandial glucose and insulin levels. *Appetite*, 55, 37–43.

Atteh, J., Onagbesan, O., Tona, K., Buyse, J., Decuypere, E., Geuns, J. (2011). Potential use of *Stevia rebaudiana* in animal feeds. *Archivos de zootecnia*, 60(229), 133-136.

Barriocanal, L., Palacios, M., Benitez, G., Benitez, S., Jimenez, J., Jimenez, N., et al. (2008). Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans, a pilot study of repeated exposures in some normatensive and hypotensive individuals and in type 1 and type 2 diabetics. Regulatory Toxicology and Pharmacology, 51, 37–41.

Bernal, J., Mendiola, J., Ibáñez, E., et Cifuentes, A. (2011). Advanced analysis of nutraceuticals. *Journal of Pharmaceutical and Biomedical Analysis*, 55, 758–774.

Bharani, A., Ganguly, A., et Bhargava, K. (1995). Salutary effect of Terminalia arjuna in patients with severe refractory heart failure. *International Journal of Cardiology*, 49, 191–199.

Blauth de Slavutzky, S. (2010). Stevia and sucrose effect on plaque formation. Journal für Verbraucherschutz und Lebensmittelsicherheit, 5, 213–216.

Brandle, J., et Telmer, P. (2007). Steviol glycoside biosynthesis. *Phytochemistry*, 68, 1855–1863.

Braz de Oliveira, A. J., Correia Gonçalves, R. A., Cantuaria Chierrito, T. P., Müller dos Santos, M., Mera de Souza, L., Gorin, P. A. J., et al. (2011). Structure and degree of polymerisation of fructooligosaccharides present in roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni. *Food Chemistry*, 129, 305–311.

Cardello, H. M. A. B., Da Silva, M. A. P. A., & Damasio, M. H. (1999). Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. *Plant Foods for Human Nutrition* (Formerly Qualitas Plantarum), 54(2), 119-129.

Chatsudthipong, V., et Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. *Pharmacology et Therapeutics*, 121,41–54.

Choudhary, K., & Bandyopadhyay, N. (1999). Preliminary studies on the inorganic constituents of some indigenous hyperglycaemic herbs on oral glucose tolerance test. *Journal of Ethnopharmacology*, 64, 179–184.

Crammer, B., et Ikan, R. (1987). Progress in the chemistry and progress of the rebaudiosides. In T. Grenby (Ed.), Developments in Sweeteners (pp. 45–64). London, UK: Elsevier Applied Science.

Edeoga, H., Okwu, D., et Mbaebie, B. (2005). Phytochemical constituents of some Nigerian medicinal plants. African *Journal of Biotechnology*, 4, 685–688.

Escudero Álvarez, E., & González Sánchez, P. (2006). La fibra dietética. *Nutrición hospitalaria*, 21, 61-72.

Jayaraman, S., Manoharan, M., et Illanchezian, S. (2008). In-vitro antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Tropical *Journal of Pharmaceutical Research*, 7, 1143–1149.

Jaworska, K., Krynitsky, A.J., Rader, J.I. (2012). Simultaneous analysis of steviol and steviol glycosides by liquid chromatography with ultraviolet detection on a mixed-mode column: application to Stevia plant material and Stevia-containing dietary supplements. *Journal of AOAC Internationa,*. 95:1588-1596.

Gardana, C., Scaglianti, M., & Simonetti, P. (2010). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener by ultra-high performance liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1217, 1463–1470.

Gasmalla, M. A. A. , Yang, R. , Musa, A. , Hua, X. , & Zhang, W. (2014). Physico-chemical Assessment and Rebauidioside A. Productively of Natural Sweeteners (*Stevia rebaudiana* Bertoni). *Journal of Food and Nutrition Research*, 2(5), 209-214.

Geuns, J. (2003). Stevioside. *Phytochemistry*, 64, 913–921.

Ghanta, S., Banerjee, A., Poddar, A., & Chattopadhyay, S. (2007). Oxidative DNA damage preventive activity and antioxidant potential of *Stevia rebaudiana* (Bertoni) Bertoni, a natural sweetener. *Journal of agricultural and food chemistry*, 55(26), 10962-10967.

Goyal, S., Samsher et Goyal, R. (2010). Stevia (*Stevia rebaudiana*) a bio-sweetener: A review. *International Journal of Food Sciences and Nutrition*, 61, 1–10.

Kaushik, R., Pradeep, N., Vamshi, V., Geetha, M., et Usha, A. (2010). Nutrient composition of cultivated Stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. *Journal of Food Science and Technology*, 47, 27–33.

Khiraoui, A., Bakha, M., Amchra, F., Ourouadi, S., Boulli, A., Al-Faiz, C., Hasib, A. (2017). Nutritional and biochemical properties of natural sweeteners of six cultivars of *Stevia rebaudiana* Bertoni leaves grown in Morocco. *Journal of Materials and Environmental Science*, 8 (3) 1015-1022.

Kim, I., Yang, M., Lee, O., et Kang, S. (2011). The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. *LWT – Food Science and Technology*, 44, 1328–1332.

Kinghorn, A., & Soejarto, D. (1985). Current status of stevioside as a sweetening agent for human use. In H. Wagner, H. Hikino, & N. Farnsworth (Eds.). *Economics and medicinal plant research*. vol. 1 (pp. 1–52). London, UK: Academic Press.

Kochikyan, V., Markosyan, A., Abelyan, L., Balayan, A., et Abelyan, V. (2006). Combined enzymatic modification of stevioside and rebaudioside A. *Applied Biochemistry and Microbiology*, 42, 31–37.

Komissarenko, N.F., Derkach, A.I., Kovalyov, I.P., Bublik, N.P. (1994). Diterpene glycosides and phenylpropanoids of *Stevia rebaudiana* Bertoni. *Rast Research* 1 (2), 53–64.

Kolb, N., Herrera, J. L., Ferreyra, D. J., Uliana, R. F. (2001). Analysis of sweet diterpene glycosides from *Stevia* *rebaudiana*: improved HPLC method. *Journal of agricultural and food chemistry*, 49(10), 4538-4541.

Kovylyaeva, G. I., Bakaleinik, G. A., Strobykina, I. Y., Gubskaya, V. I., Sharipova, R. R., Al'Fonsov, V. A., … & Tolstikov, A. G. (2007). Glycosides from *Stevia rebaudiana*. Chemistry of Natural Compounds, 43(1), 81-85.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry*, 27(4): 969–78.

Lavini, A., Riccardi, M., Pulvento, C., De Luca, S., Scamosci, M., d'Andria, R., (2008). Yield, Quality and Water Consumption of *Stevia rebaudiana* Bertoni Grown under Different Irrigation Regimes in Southern Italy. *Italian Journal of Agronomy* 2, 9.

Lemus-Modaca, R., Vega-Gálvez, A., Zura-Bravo, L., Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*., 132, 1121–1132.

Li, G., Wang, R., Quampah, A. J., Rong, Z., Shi, C., & Wu, J. (2011). Calibration and prediction of amino acids in stevia leaf powder using near infrared reflectance spectroscopy. *Journal of agricultural and food chemistry*, 59(24), 13065-13071.

Liu, J. C., Kao, P. K., Chan, P., Hsu, Y. H., Hou, C. C., Lien, G. S., Hsieh, M. H., Chen, Y. J., Cheng, J. T. (2003). Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. *Pharmacology*, 67, 14–20.

Makapugay, H., Nanayakkara, N., et Kinghorn, A. (1984). Improved highperformance liquid chromatographic separation of the *Stevia rebaudiana* sweet diterpene glycosides using linear gradient elution. *Journal of Chromatography*, 283, 390–395.

Mohammad, M., Mohammad, U., Sher, M., Habib, A., et Iqbal, A. (2007). In vitro clonal propagation and biochemical analysis of field established *Stevia rebaudiana* Bertoni. *Pakistan Journal of Botany*, 39, 2467–2474.

Mishra, P., Singh, R., Kumar, U., et Prakash, V. (2010). *Stevia rebaudiana* – A magical sweetener. Global Journal of Biotecnology et Biochemistry, 5, 62–74.

Muanda, F. N., Soulimani, R., Diop, B., & Dicko, A. (2011). Study on chemical composition and biological activities of essential oil and extracts from *Stevia rebaudiana* Bertoni leaves. *LWT-Food Science and Technology*, 44(9), 1865-1872.

Pól, J., Hohnová, B., et Hyötyläinen, T. (2007). Characterization of *Stevia rebaudiana* by comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. *Journal of Chromatography* A, 1150, 85–92.

Prosky, L. (2001). What is dietary fibre? A new look at the definition. *Advanced dietary fibre technology*, 63-73.

Sankhala, A., Sankha, A. K., Bhatnagar, B., & Singh, A. (2005). Nutrient composition of less familiar leaves consumed by the tribals of Udaipur region. *Journal of Food Science and Technology-mysore*, 42(5), 446-448.

Savita, S., Sheela, K., Sunanda, S., Shankar, A., et Ramakrishna, P. (2004). *Stevia rebaudiana* – A functional component for food industry. *Journal of Human Ecology*, 15, 261–264.

Segura-Campos, M., Barbosa-Martín, E., Matus-Basto, Á., Cabrera-Amaro, D., Murguía-Olmedo, M., Moguel-Ordoñez, Y., & Betancur-Ancona, D. (2014). Comparison of chemical and functional properties of *Stevia rebaudiana* (Bertoni) varieties cultivated in Mexican Southeast. *American Journal of Plant Sciences*, 5(3), 286.

Serio, L. (2010). La *Stevia rebaudiana*, une alternative au sucre. *Phytothérapie*, 8, 26–32.

Sivaram, L., Mukundam, U. (2003). In vitro culture studies on *Stevia rebaudiana*. In Vitro Cellular and Developmental Biology – Plant, 39, 520–523.

Shock, C. (1982). Experimental cultivation of Rebaudi's stevia in California. University of California – Davis, *Agronomy Progress Report*, April pp. 122.

Shukla, S., Mehta, A., Bajpai, V. K., & Shukla, S. (2009). In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. *Food and Chemical Toxicology*, 47(9), 2338-2343.

Shukla, S., Mehta, A., Mehta, P., & Bajpai, V. K. (2012). Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. *Experimental and Toxicologic Pathology*, 64(7), 807-811.

Soejarto, D. (2002). Botany of Stevia and *Stevia rebaudiana*. In A. Kinghorn (Ed.), Stevia: The genus Stevia (pp. 18–39). London, New York: Taylor and Francis.

Tadhani, M., et Subhash, R. (2006a). Preliminary studies on *Stevia rebaudiana* leaves: Proximal composition, mineral analysis and phytochemical screening. *Journal of Medical Sciences*, 6, 321–326.

Tadhani, M. B., Patel, V. H., & Subhash, R. (2007). In vitro antioxidant activities of *Stevia rebaudiana* leaves and callus. *Journal of Food Composition and Analysis*, 20(3), 323-329.

Thomas, J., & Glade, M. (2010). Stevia: It's not just about calories. *The Open Obesity Journal*, 2, 101–109.

Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., de Bruyne, T., et al. (1999). Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo.

Phytomedicine, 6, 59–66.
Wallin, H. (2007). Steviol glycosides. 63rd Joint FAO/WHO Expert Committee on Food Additives (JECFA) – *Chemical and Technical Assessment* (CTA) (pp.1–8).
Wölwer-Rieck, U. (2012). The leaves of *Stevia rebaudiana* (Bertoni), their constituents and the analyses thereof: a review. *Journal of agricultural and food chemistry*, 60(4), 886-895.
Zeng, J., Cai, W., Yang, W., & Wu, W. (2013). Antioxidant abilities, phenolics and flavonoids contents in the ethanolic extracts of the stems and leaves of different *Stevia rebaudiana* Bert lines. *Sugar Tech*, 15(2), 209-213.