J. Sylhet Agril. Univ. 4(1):15-25, 2017 ISSN: 2308-1597

REVIEW ON *STEVIA REBAUDIANA* AS A NON-CALORIC NATURAL SWEETENER PRODUCING PLANT

S R Ahmed* and S Mukta

Department of Plant and Environmental Biotechnology, Sylhet Agricultural University, Sylhet-3100

(Available online at: www.jsau.com.bd)

Abstract

Stevia rebaudiana, a medicinal plant, is a darling gift of nature. It is well-aquainted for its non-caloric bio-sweeteners and medicinal value. Previous studies have shown that *Stevia* leaf contains phytochemicals which has therapeutic values such as antioxidants, antimicrobials, antidiabetics, antihypertensive, cardiotonic etc. Human nutritions exist in great content in *Stevia* leaves owing to its good proximate composition, mineral contents and health-promoting phytochemical constituents. Various extraction methods and techniques were used to extract, purify, characterize and analyze natural herbal sweetener, stevioside and rebaudioside, which are the chief components of sweetness. Leaves of *Stevia* are enriched with high content of protein, carbohydrate, some essential minerals, all indispensible amino acids except tryptophan, folic acid, vitamin C and some bioactive chemical constituents as well as palmitic and linolenic acids. Both stevioside and rebaudioside are metabolized by intestinal microorganism. Rebaudioside is transformed to steviol and finally it produces glucose and a molecule of steviol. Different scientific reports suggested no adverse side effects observed in taking stevia powder as sweeteners. Many suitable protocols have been established for *in vitro* regeneration of the sweetener plant *Stevia*. This review article flourishes the collection of basic data on *Stevia* plant to present the beneficial role of *Stevia* and its metabolites on health promoting properties.

***Keywords*:** *Stevia*, leaves, sweetener, stevioside, phytochemicals.

Introduction

Nature has bestowed our world with an enormous wealth of medicinal plants. Medicinal plants have been recognized as potential drug candidates. *Stevia*, a natural sweetener producing medicinal plant having nutritional, thearapeutic and industrial importance is being used all over the world. *Stevia rebaudiana* (Bertoni) is a herbaceous perennial plant (2n=22). Though there are above 200 species of the genus *Stevia*, only *S. rebaudiana* gives the sweetest essence (Savita *et al*., 2004; Singh and Rao, 2005). Leaves of *Stevia* produce diterpene glycosides (stevioside and rebaudiosides), non-nutritive, non-toxic, high-potency sweeteners and may replace sucrose as well as other artificial sweetners, being 300 times sweeter than sucrose (Yadav *et al.*, 2011). *S. rebaudiana* leaves are commonly referred to as honey leaves, candy leaves and sweet leaves. This is due to the production of stevoil glycosides sweetening compounds (Brandle and Telmer, 2007). 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). 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. This review also aims for a better understanding and acceptance of *Stevia* as a suitable raw material for human diet.

Botanical description

It is estimated that there are over 250 species of *Stevia* which grows wild around the world. However sweetening properties have been found in *Stevia rebaudiana* and in some species. *Stevia* is a short day plant that grows up to 1 m tall. It has sessile, elliptic, 3-4 cm long leaves. The root system of the plant is extensive; the stem is woody and weak-pubescent at the bottom. It has white flowers with a pale purple throat. They are small in size and arranged in the form of small corymbs (Goettemoeller and Ching, 1999; Singh and Rao, 2005).

Propagation

Stevia can be propagated using the seed, or stem cutting. Propagation of *Stevia* plant is restricted due to low fertility of seeds coupled with very low germination rate (Tadhani *et al*., 2006; Yang *et al*., 1981). Seed does not produce a

***Corresponding author: SR Ahmed,** Department of Plant and Environmental Biotechnology, Sylhet Agricultural University, Sylhet-3100, Bangladesh, Email: rashel.peb@sau.ac.bd*

homogenous population. On the other hand, vegetative propagation is limited by low number of individuals that can be obtained from single plant (Yang *et al*., 1981; Sivaram and Mukundan, 2003). In addition, due to the low primary growth, the seedling is not able to compete with weeds (Jain *et al.,* 2009). Keeping these difficulties in consideration, propagation through tissue culture could be suitable as an alternative method to obtain sufficient number of plants with homogeneous population within short period of time (Ibrahim *et al*., 2008). There are various reports of *in vitro* propagation of *Stevia* using different explants (Akita *et al.,* 1994; Salim *et al.,* 2006 and Uddin *et al*., 2006). Ghauri *et al*. (2009) reported the micro propagation from apical meristem and nodal segment. Some of the reports clearly support the possibility of propagating *S. rebaudiana* by tissue culture techniques (Razak *et al*., 2014; Uddin *et al*., 2006; Pande and Gupta, 2013). *In vitro* clonal propagation of *Stevia* was carried by using leaf, nodal, inter nodal segment and shoot tip as explant (Das *et al*., 2006; Preethi *et al*., 2011; Uddin *et al*., 2006; Rao *et al.,* 2012; Anbazhagan *et al.,* 2010 and Giridhar *et al*., 2010).

Diterpene Glycosides, Stevioside and Rebaudioside

Diterpene glycosides are responsible for its high sweetening potential of leaves. The bio-sweeteners of *Stevia* leaves, called steviol glycosides, are isolated and identified as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside (Geuns, 2003). Stevioside was found to be the most ample stevia glycoside (4–13% w/w) 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). Stevioside accounts for 4 up to 13% all glycosides in *Stevia*. It is bittery or stringent when it is tasted. Comparative organoleptic analyses showed that pure stevioside is 300 times more sweeter than sucrose at a concentration of 0.4% (Hojden, 2000). Kroyer (2010) reported that steviosides are stable at various processing and storage conditions. Rebaudioside A is 250–450 times more sweeter than sucrose and it is found in *Stevia rebaudiana* at 2–4% leaf dry matter. It is the most stable of glycosides and has no bitter after taste, in contrast to steviosides. Rebaudioside A is metabolised by intestinal microorganisms to stevioside and finally it is transformed to glucose and a molecule of steviol. Apart from diterpene glycosides, sweet leaf contains also labdanediterpenes and triterpenes (Marcinek and Krejpcio, 2015).

Extraction, Isolation and Purification of Stevioside

Stevia sweetener (Stevioside) was extracted from the dried ground leaves of *Stevia* plant by using various solvent and techniques. Researchers isolated, purified and characterized sweet Stevioside and Rebaudioside from *Stevia* leaves by using different procedures are as follows-

- Afandi *et al.* (2013) developed a extraction method consisted of solvent extraction of leaf powder using various solvents like petroleum ether, methanol, diethyl ether and butanol followed by its purification using high performance liquid chromatography in order to obtain bioactive compound rebaudioside.
- Kaur *et al*. (2014) extraceted and characterized steviosides which involved four main steps: extraction into polar organic solvent, decolourization, coagulation or concentration, column chromatography and crystallization.
- Rao *et al*. (2012) established a new process of extraction of steviosides from the *Stevia* leaves in which the dry leaves were grounded, defatted, and extracted through pressurized hot water extractor (PHWE), followed by purification and concentration of the sweet glycosides through ultra (UF) and nano (NF) membrane filtration in obtaining high (98.2%) purity steviosides.
- Deshmukh and Kedari (2014) used water, methanol and ethanol extraction methods to extract *Stevia* sweetener (Stevioside) from the dried ground leaves of *Stevia* plant. Further it was purified and recovered using Calcium hydoxide and Ion exchange chromatography.
- Pasquel *et al*. (2001) extracted steviol glycoside with carbon dioxide and water or with the use of CO_2, water and ethanol.
- Liu *et al*. (1997) developed a simple, efficient subcritical fluid extraction (SubFE) method to extract steviol glycosides in *Stevia rebaudiana*.
- Tambe *et al*. (2010) isolated, purified, analyzed and characterized stevioside by using various chromatographic and analytical methods including TLC, UV spectroscopy, Fourier Transform Infra-Red

Spectrophotometer (FTIR), NMR spectroscopy and High performance liquid chromatography (HPLC) methods and confirmed its structure (Fig. 1) as follows-

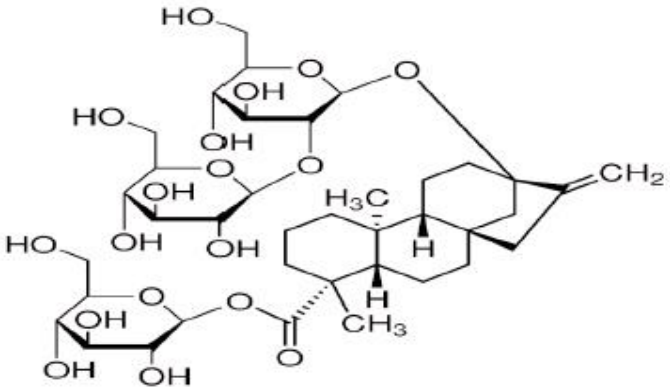

Fig. 1. Structure of stevioside

Phytochemical constituents

Plants accumulate secondary metabolites called phytochemicals to defend themselves against microbial infections or infestations by pests. Phytochemicals are active ingredients which possess therapeutic properties that are considered as a medicine or drug (Shakya, 2016). Shukla *et al.* (2013) showed the presence of different phytochemicals in *Stevia* leaves extract with their respective solvent systems. The phytochemical properties of bioactive chemicals present in *Stevia* leaves are summarized in the Table 1.

Table 1. List of some plant active components and their pharmacological properties

Phytochemicals	Pharmacological properties	References
Phenols	anti-inflammatory, anti-apoptotic and anti-ageing properties of the plant	Archana *et al.*, 2012
Saponins	foaming and surface active agents; applied in detergents; pesticides and molluscicides; antibacterial agents; used to treat hypercholesterolemia, hyperglycemia and obesity	Archana *et al.*, 2012; Mohanta *et al*., 2007
Flavonoids	antimicrobial, anti-inflammatory, antiallergic, anticancer, antineoplastic activity, free radical scavenging activity, prevent oxidative cell damage and used for the treatment of intestinal disorders	Archana *et al.*, 2012; Kam and Liew, 2002; Rio *et al*., 1997; Salah *et al.*, 1995
Alkaloids	pain-removing medication	Cushnie and Lamb, 2006
Tannins	healing properties of wounds, inflamed mucous membranes, ulcerated tissues and used in treating intestinal disorder such as diarrhea and dysentery	Rio *et al.*, 1997; Ranjan *et al.*, 2013.
Steroids	cholesterol-reducing properties and also helps in regulating the immune response	Shah *et al.*, 2009
Cardiac glycosides	inhibiting properties on Na+ and K+ pump that increase the availability of sodium ions and calcium ions to heart muscles to improve cardiac output and reduce heart distension; used in the treatment of congestive heart failure and heart arrhythmia.	Schneider and Wolfling, 2004
Coumarins	antimicrobial and anti-inflammatory effects and beneficial for hyper proliferative skin diseases	Theis and Lerdau, 2003

Nutritional Aspect of *Stevia* Leaves

Savita *et al.* (2004) analysed *Stevia* leaves on a dry weight basis and calculated an energy value of 2.7 kcal g^{-1}. Six sweetening compounds have been reported in the leaves of *S. rebaudiana* Bertoni—stevioside, rebaudiosides A, D and E, dulcosides A and B (Kohda *et al.*,1976; Kobayashi *et al.*, 1977). This means that *Stevia* may be granted the status of a low calorie sweetener; the benefits associated to *Stevia* leaf are mainly due to their nutritional composition which is a good source of carbohydrates, protein and crude fibred that promotes wellness and reduces the risk of certain diseases. Degree of processing brings about changes in chemical composition of *Stevia* leaves. Leaf drying method affects the chemical composition of this plant (Gasmalla *et al.*, 2014). Fat content in dry matter of *Stevia* leaves make up to 1.9–4.34 g 100 g^{-1} DM (Abou-Arab *et al.*, 2010; Siddique *et al.*, 2014). Protein and carbohydrate contents in dry weight basis of *Stevia* leaves ranges from 10.0 to 18.0 and 52 to 64.06, respectively (Srivastava *et al.*, 2016; Maira Segura-Campos *et al.*, 2014; Mishra *et al.*, 2010; Abou-Arab *et al.*, 2010). The proximate composition of *S. rebaudiana* Bertoni that is presented in Table 2.

Table 2. Proximate chemical analysis of dried Stevia leaves (g 100 g^{-1} dry weight basis)

Parameter	References						
	Srivastava *et al.* (2016)	**Shuvo *et al.* (2015)**	**Maira Segura-Campos *et al.* (2014)**	**Gasmalla *et al.* (2014)**	**Atteh *et al.* (2011)**	**Mishra *et al.* (2010)**	**Abou-Arab *et al.* (2010)**
Moisture	6.7	6.32	7.8	10.73	ND	7.0	5.37
Ash	11.5	7.05	11.93	12.06	15.5	11	7.41
Fat	4.2	3.55	3.04	6.13	2.6	3	3.73
Protein	18.0	15.13	15.05	13.68	16.0	10	11.4
Carbohydrates	30.4	53.52	64.06	63.10	ND	52	61.9
Crude fiber	14.89	10.5	5.92	5.03	6.8	18	15.5
Reducing sugar	ND	3.62	ND	4.50	ND	ND	ND

ND=Not determined

Carbohydrates are the chief sources of energy. The presence of fructooligosaccharides and polysaccharides in sweet leaf, roots and leaves regulate lipid metabolism and control blood sugar level (Braz De Oliveira *et al.*, 2011). Proteins, are string of amino acids, serve as essential cell components. The content of the essential amino acids determine the protein quality of a food (Latham, 2002). Mohammad *et al.* (2007) characterized nine indispensible amino acids in *Stevia* leaves, namely glutamic acid, aspartic acid, lysine, serine, isoleucine, alanine, proline, tyrosine and methionine with the exception of tryptophan, whereas Abou-Arab *et al.* (2010) isolated 17 amino acids. According to Periche (2014), the total concentration of the eleven amino acids found was 11.70 mg g^{-1} in dried leaves and from 6.84 to 9.11 mg g^{-1} of *Stevia* in infusions. Amino acids with its quantity are enlisted in Table 3.

Table 3. Amino acid composition of *Stevia* leaves (g 100 g^{-1} dry matter)

Amino acid (Essential amino acid)	References	
	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	2.30
Methionine	1.45	ND
Valine	0.64	0.94
Threonine	1.13	0.75
Isoleucine	0.42	0.72

ND=Not determined

Very small amounts of some mineral elements are needed in human diets, but are vital for metabolic purposes, and are thus called essential trace elements (Latham, 2002). The main elements are sodium, magnesium, phosphorus, sulphur, chlorine, potassium, and calcium which are classified as macroelements and the minor elements are chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine (Adotey *et al.*, 2009). They are involved in all aspects of growth, health and reproduction, participating also in the formation of cells, tissues and organs (Szefer and Nriagu, 2007). Data obtained by Kobus-Moryson *et al.* (2014) showed that considerable amounts of Zn and Cu are present in the extract of honey-leaf. Content of minerals in *Stevia* leaves is represented in Table 4.

Table 4. Minerals content of dried *Stevia rebaudiana* leaves (mg 100 g^{-1} dry base of extract)

Parameter	Amount (mg 100 g^{-1})		
	Shuvo *et al.* (2015)	Goyal and Samsher (2010)	Mishra *et al.* (2010)
Iron (Fe)	34.2	3.9	55.3
Sodium (Na)	184.3	89.2	190
Potassium (K)	2500	1780	1800
Calcium (Ca)	534.43	544	464.4
Magnesium (Mg)	465.34	349	349
Phosphorus (P)	304.7	318	11.4
Chloride (Cl)	49.6	ND	ND
Zinc (Zn)	ND	1.5	1.5

ND=Not determined

In the leaf oil of *Stevia*, Tadhani and Subhash (2006) identified six fatty acids (Table 5) using methyl ester standards. Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were recognized in the leaf oil. Among the identified fatty acids, palmitic acid content was found to be highest, whereas stearic acid content was least (Table 5). *Stevia* leaf oil proves to be a rich source of linolenic acid. However, infusions showed higher levels of certain amino acids (alanine, asparagine, leucine and proline). Sweet leaves of *Stevia* are a source of folic acid, ascorbic acid and slight amounts of vitamins B (Bugaj *et al.*, 2013). Kim *et al.* (2011) isolated water-loving vitamins and reported the quantity of water-soluble vitamins from leaves and calluses of sweet leaf (Table 6).

Table 5. The composition of fatty acids in oil from *Stevia* leaves

Fatty acid	Fatty acid contents, g 100 g^{-1} dry base of extract	
	Tadhani and 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
Linoleic acid	12.40	16.8
Linolenic acid	21.59	36.2

Table 6. Water-soluble vitamins of *Stevia rebaudiana* leaves (mg 100 g^{-1} dry base of extract)

Vitamins	References
	Kim *et al.* (2011)
Vitamin C	14.97
Vitamin B2	0.43
Vitamin B6	0.00
Folic acid	52.18
Niacin	0.00
Thiamine	0.00

Pharmacological aspect of *Stevia*

Leaves of *Stevia* showed some important pharmacological activities of the plant such as hypotensive, heart tonic action, antidiabetic, antimicrobial, antioxidant, anti-inflammatory, antihypertensive and anticancer.

Antihypertensive activity

Chan *et al.* (2000) evaluated the effect of stevioside in human hypertension by performing a multi-centre, randomized, double blind, and placebo-controlled study on 106 Chinese hypertensive subjects (men and women) with diastolic blood pressure between 95 and 110 mmHg and ages were ranging from 28 to 75 years. Their study revealed that the systolic and diastolic blood pressure decreased significantly and the effect persisted during the whole year. No significant changes were observed in blood biochemistry parameter including lipid and glucose. Additionally no adverse effects were observed. Hsieh *et al.* (2003) investigated the long-term (2-year) efficacy and tolerability of stevioside in patients with mild essential hypertension. It may be regarded as an alternative or supplementary therapy for patients with hypertension. A study of Lee *et al.* (2001) revealed the inhibitory effect of stevioside on calcium influx against hypertensive. Liu *et al.* (2003) also reported that stevioside possesses antihypertensive activity and its hypotensive mechanism was due to inhibition of the Ca^{2+} influx.

Antidiabetic activity

Sweet leaf reduces blood glucose, ALT and AST, and increases insulin level in rat. Streptozotocin is a compound that induces diabetes in rat. *Stevia* leaves could protect rats against streptozotocin induced diabetes. It also reduces the risk of oxidative stress and ameliorates liver and kidney damage (Shivanna *et al.*, 2013). *Stevia* leaf powder influenced blood sugar levels both Fasting blood sugar (FBS) and Post prandial (PPBS) blood sugar positively (Mishra, 2011).

Antibacterial and antifungal activity

Plants have provided a source of inspiration for novel drug compounds to many scientists. Scientists used different solvent extracts (methanol, ethanol, ethylacetate, acetone, petroleumether, chroloform) to investigate the antimicrobial activity of *Stevia* leaves (Ghosh *et al*., 2008). *Stevia* is thought to inhibit the growth of certain bacteria and other infectious organisms (Patil *et al.*, 1996; Sivaram and Mukundam, 2003). In some antimicrobial activity screening studies, these extracts exhibited susceptibility enough to inhibit the growth of certain pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Aeromonas hydrophila* (Ghosh *et al.*, 2008; Debnath, 2008; Jayaraman *et al.*, 2008; Tadhani and Subhash, 2006). Antifungal activity was observed against *Aspergillusniger*, *Penicillium chrysogenum*, *Alternariasolani* (Ghosh *et al.*, 2008). *Fusariumoxysporum* showed maximum zone of inhibition by methanolic plant extracts of *Stevia rebaudiana* in the study of Arya *et al.* (2012). Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Jayaraman *et al.*, 2008). The presence of phytochemicals in leaves might have contributed to the anti-bacterial activity (Rajendran and Sundararajan, 2010).

Antioxidant activity

Free radicals are considered to be the causative agents in the development of neurological diseases, inflammations, reduced immunity, ageing, ischaemic heart disease, stroke, Alzheimer's and Parkinson's disease as well as cancer (Hou *et al.*, 2003; Parejo *et al.*, 2002). Leaves of *Stevia rebaudiana* were reported to contain polyphenolic compounds having antioxidant properties (Table 7) (Muanda *et al.*, 2011; Shukla*et al.*, 2009; Tadhani *et al.*, 2007). Antioxidants are compounds that have gained importance in recent years due to their ability to block the action of free radicals (Devasagayam *et al.*, 2004). Varieties of antioxidants were obtained from the extracts of *Stevia rebaudiana*, they include, opigenin, kaempferol and quereitrin that inhibited DNA strand damage (Ghanta *et al.*, 2004 and Stoyanova *et al.*, 2011). Phenolic compounds have antioxidant activity. Contents of flavonoid and other phenolic substance act against the development of cancer and heart disease (Kahkonen *et al.*, 1999). Antioxidants are compounds that interact with and neutralize free radicals. 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 measure the antioxidant activity. 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 *et al.*, 2007; Evelson *et al.*, 2001; Ou *et al.*, 2001; Benzie and Strain, 1996). 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 *et al.*, 2010), where the antioxidant activity is determined as the

percentage inhibition of the DPPH free radical (Turkmen *et al.*, 2005). 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). Total phenolic compounds, flavonoids, tannins and antioxidant activity of leaves extract of *Stevia rebaudiana* with references are presented in Table 7.

Cardiovascular action

It is used as a cardio tonic to normalize blood pressure levels, regulate heart beat, and for other cardiopulmonary indication were 1st reported in the rat studies in 1978 (Adesh *et al*., 2012).

Antitumor activity

An antitumor study of stevioside was examined and stevioside suppressed 12-O tetradecanoylphorbol-13-acetate (TPA) induced tumor promotion in a skin carcinogenesis in mice (Nakamura *et al.*, 1995). Jayaraman *et al.* (2008) used MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to evaluate cytotoxicity based on metabolic reduction of MTT.

Table 7. Total phenolic compounds, flavonoids, tannins and antioxidant activity of leaves extract of *Stevia rebaudiana*

Parameter	References			
	Singh *et al.* (2012)	**Kumari *et al.* (2016)**	**Gawel-Bęben *et al.* (2015)**	**Mandal and Madan (2013)**
Phenols	11.04 (mg GAE g^{-1} DW)	12.44 (mg GAE g^{-1} DW)	AE:3.85 (mg GAE g^{-1} EL) EE:7.65 (mg GAE g^{-1} EL)	5.67 (mg GAE L^{-1} of Bangalore variety extract)
Tannins	12.98 (mg TAE g^{-1}DW)	ND	ND	ND
Flavonoids (% Quercetin)	2.73	29.33 (mg RE g^{-1} DW)	AE=2.03 (mg Qug^{-1} EL) EE=3.85(mg QE g^{-1} EL)	52.87 (mg QE L^{-1} of Bangalore variety extract)
Total antioxidant activity	ND	27.67 (mg TE g^{-1} DW)	ND	ND

ND=Not determined
TE=Trolox equivalent; GAE=Gallic acid equivalent; TAE=Tannic acid equivalent; RE=Rutin equivalent; QE=Quercetin equivalent; DW=Dry weight; AE=Aqueous extrac;, EE=Ethanolic extract; EL=Extracted leaves

Acetone extracts of *Stevia rebaudiana* is non-toxic to the normal cells and also has both anticancer and anti-proliferative activities against the cancerous cells (Jayaraman *et al.*, 2008). Leaf extract of *Stevia* contains a compound named Labdanesc lareol which has anti-tumorous and cytotoxic properties (Kaushik *et al.*, 2010).

Anthelmintic activity

Shukla *et al.* (2013) investigated the leaves of *Steviar ebuadiana* for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli* worms. Water extract showed significant anthelmintic activity.

Conclusion

Stevia is used extensively as a non-caloric sugar substitute. It has good proximate composition and health-promoting phytochemical constituents. For having functional and sensory properties to those of other high potency sweeteners, *Stevia* powder can be used as 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 important amino acids which are conducive to human health. Studies have reported the health promoting benefits of this natural herb *Stevia* which is well known as therapeutic agent and an efficient medication for curing chronic diseases. *Stevia* leaves are effective natural medication to develop new lead compound by clinical investigation. Further research needs to be conducted to investigate interactions of *Stevia* metabolites with food components and to determine maximum daily intake of this food additive.

References

Adesh A B, Gopalakrishna B, AkkiKusum S and Tiwari O P. 2012. An Overview on Stevia: A Natural Calorie Free Sweetener. Int. J. Adv. Pharma., Biol. & Che. 1(3):362-368.

Abou-Arab A, Abou-Arab A, Abu-Salem M F. 2010. Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana*Bertoni plant. Afr. J. Food Sci. 4:269–281.

Adotey D, Serfor-Armah Y, Fianko J and Yeboah P. 2009. Essential elements content in core vegetables grown and consumed in Ghana by instrumental neutron activation analysis. Afr. J. Food Sci. 3:243–249.

Afandi A, Sarijan S and Shaha R K. 2013. Optimization of Rebaudiosidea Extraction from *Stevia rebaudiana* (Bertoni) and Quantification by High Perfomance Liquid Chromatography Analysis. J. Tropi. Resources and Sustainable Sci. 1(1):62-70.

Ahmad N, Fazal H, Abbasi and 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 J. Phytomedicine. 2:231–239.

Akita M, Shigeoka T, Koizumi Y and Kawamura M. 1994.Mass propagation of shoots of *Stevia rebaudiana* using a large scale bioreactor.Plant Cell Reports. 13:180–183.

Anbazhagan M, Dhanavel D, Kalpana M, Natarajan V and Rajendran R. 2010.*In vitro* propagation of *Stevia Rebaudiana* Bertoni Emir. J. Food Agric. 22:216-222.

Archana P, Samatha T, Mahitha B, Chamundeswari and Ramaswamy N. 2012. Preliminary phytochemical screening from leaf and seed extracts of *Sennaalata*L. Roxb-an Ethno medicinal plant. International J. Pharma. and Biol. Research. 3:82-89.

Arya A, Kumar S and Kasana M S. 2012.*In vitro* regeneration of *Stevia* and evaluation of antimicrobial and antiprotozoal properties of regenerated calli and plants. Electronic J. Plant Breeding. 3(3):916-924.

Atteh J, Onagbesan O, Tona K, Buyse J, Decuypere E and Geuns J. 2011.Potential use of *Stevia rebaudiana*in animal feeds. Arch. Zootec. 60:133–136.

Benzie I F F and Strain J J. 1996. The ferric reducing ability of plasma as a measure of ‘‘antioxidant power’’: the FRAP assay. Anal.Biochem. 239:70–76.

Brandle J E and Telmer P G. 2007.Steviol glycoside biosynthesis. Phytochemistry. 68:1855-1863.

Braz-De-Oliveira A, Goncalves R A C, Chierrito T P C, Souza L, Gorin P A J, Sassaki G L and Iacomini M. 2011. Structure and degree of polymerisation of fructooligosaccharides present in roots and leaves of *Stevia rebaudiana* Bertoni. Food Chem. 129:305–311.

Bugaj B, Leszczynska T, Pysz M, Kopec A, Pacholarz J and Pysz-Izdebska K. 2013. Charakterystyka i właściwości prozdrowotne *Stevia rebaudiana* Bertoni [Characteristics and health-promoting properties of *Stevia rebaudiana* Bertoni]. Żywn. NaukaTechn. Jakość. 3(88):27–38.

Cacciola F, Delmontea P, Jaworska K, Dugo P, Mondello L and Rader J. 2011. Employing ultra high pressure liquid chromatography as the second dimension in a comprehensive two-dimensional system for analysis of *Stevia rebaudiana* extracts. J. Chromat. 1218:2012–2018.

Chan P, Tomlinson B, Chen Y, Liu J, Hsieh M and Cheng J T. 2000. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Clin. Pharmacol. 50:215-220.

Cushnie T P and Lamb A J. 2006. Antimicrobial activity of flavanoids. Int J. Antimicrob Agents. 27(2):181.

Das K, Dang R and Rajasekharan P E. 2006. Establishment and maintenance of callus of *Stevia rebaudiana* Bertoni under aseptic environment. Nat. Prod. Rad. 5(5):373-376.

Deshmukh S R and Kedari V R. 2014. Isolation, purification and characterization of sweetners from *Stevia rebaudiana* (bertoni) for their anticancerous activity against colon cancer World J. Pharm. and Pharma. Sci. 3(5):1394-1410.

Devasagayam T P, Tilak J, Boloor K K, Sane K S, Ghaskadbi S S and Lele R D. 2004. Free radicals and antioxidants in human health: current status and future prospects. J. Assoc. Physicians India. 52:794-804.

Evelson P, Travacio M and Repetto M. 2001. Evaluation of total reactive antioxidant potential of tissue homogenates and their cytosols. Archives Biochem. Biophys. 388:261–266.

Gasmalla M A A, Yang R, Issoufou-Amadou and Hua X. 2014. Nutritional Composition of *Stevia rebaudiana* Bertoni Leaf: Effect of Drying Method. Trop. J. Pharm. Res.. 13 (1):61-65.

Gaweł-Bęben K, Tomasz Bujak T, Nizioł-Łukaszewska Z, Antosiewicz B, Jakubczyk A, Monika Karaś M and Rybczyńska K. 2015. *Stevia rebaudiana* Bert. Leaf Extracts as a Multifunctional Source of Natural Antioxidants. Molecules. 20:5468-5486.

Geuns J. 2003. Stevioside. Phytochemistry. 64:913–921.

Ghanta S, Banerjee A, Poddar A and Chattopadhyay S. 2007. Oxidative DNA damage preventive activity and antioxidant potential of *Stevia rebaudiana* (Bertoni) Bertoni: A natural sweetener. J. Agri. & Food Chem. 26:10962-10967.

Ghosh S, Subudhi E and Nayak S. 2008. Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. Int. J. Integrat. Biol. 2(1):27-31.

Ghuari E G, Fatima A, Ghani S and Shafi N. 2009. Micropropagation and antimicrobial activity of anexotic sweetener plant (*Stevia rebaudiana*Bertoni). Pak. J. Biochem. Mol. Biol. 42(2):53-56.

Giridhar P, Sowmya K S, Ramakrishna A and Ravisshankar G A. 2010. Rapid clonal propagation and stevioside profile of stevia *rebaudiana* Bertoni. Int. J. Plant Dev. Biol. 4(1):47-52.

Goettemoeller J and Ching A. 1999.Seed germination in *Stevia rebaudiana*. Prerspectives on new crops and new users. J Janick (Ed.), ASHS Press. Alexandria, VA.

Goyal S and Samsher R. 2010. *Stevia* a bio-sweetener: a review. Inter. J. Food Scienc. and Nutr. 61:1–10.

Haider-Ali, Ahmed H, Marwat K B and Lu C. 1993. The use of Thidiazuran in tissue culture. *In vitro* cell development. Biol. Plant. 29:92-96.

Hojden B. 2000. *Stevia rebaudiana* source of natural sweeteners. Wiad. Ziel.1–3.

Hou W C, Lin R D, Cheng K T, Hung Y T, Cho C H, Chen C H, Hwang S Y and Lee M H. 2003. Free radical scavenging activity of Taiwanese native plants. Phytomedicine.10: 170–175.

Hsieh M H, Chan P, Sue Y M, Liu J C, Liang T H and Huang T Y. 2003. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. Clin. Ther. 25:2797-2808.

Ibrahim I A, Nasr M I, Mohammed B R and Zefzafi M M E L. 2008. Nutrient factors affecting *in vitro* cultivation of *Stevia rebaudiana*. Sugar Tech. 10(3):248-253.

Jain P, Kachhwaha S, Kothari S L. 2009. Improved micropropagation protocol and enhancement in biomass and chlorophyll content in *Stevia rebaudiana* Bertoni by using high copper levels in the culture medium. Scientia Horticulturae. 119:315–319.

Jayaraman S, Manoharan M S and Illanchezian S. 2008. In-vitro Antimicrobial and Antitumor Activities of stevia *Rebaudiana* (Asteraceae) Leaf Extracts.Tropical J. Pharm. Res. 7(4):1143-1149.

Kahkonen M P, Hopia A I, Vuorela H J, Rauha J P, Pihlaja K, Kujala T S and Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agri. and Food Chem. 47:3954–3962.

Kam P C A and Liew. 2002. Traditional Chinese Herbal medicine and anesthesia. Anesthesia. 57(11):1083-1089.

Kaur G, Pandhair V and Cheema G S. 2014.Extraction and characterization of steviol glycosides from *Stevia rebaudiana* bertoni leaves. J. Med. Plants Studies. 2(5):41-45.

Kaushik R, Pradeep N, Vamshi V, Geetha M and 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. J. Food Sci. and Technol. 47:27–33.

Kim I, Yang M, Lee O and Nam-Suk K. 2011. The antioxidant activity and the bioactive compound content of stevia rebaudiana water extracts. L W T-food Sci. Technol. 44: 1328-1332.

Kim J, Choi Y H and Choi Y H. 2002. Use of stevioside and cultivation of Stevia rebaudiana in Korea. In: kinghorn,A.D. (Ed.), Stevia, the Genus Stevia. Medicinal and Aromatic Plants-Industrial profiles, Taylor and Francis, London and NY. 19:196–202.

Kobayashi M, Horikawa S, Degrandi I H, Veno j and Nijisuhasi H. 1977. Facts of Stevia. Phytochemistry. 16: 1405-1407.

Kobus-Moryson M, Gramza-Michalowska A, Kobus-Cisowska J and Korczak J. 2014. Zawartoscwybranychierwiastkow w ekstraktachstewii (Stevia rebaudiana Bertoni) [contents of selected elements in extracts from sweet leaf (stevia rebaudiana Bertoni)]. Probl. Hig. Epidemiol. 95(2):445-448.

Kohda H, Kasai R, Yamasaki K, Murakami K and Tanaka P. 1976. Steviodsides from Stevia rebaudiana Bertoni. Phytochemistry. 15:981-982.

Kroyer G. 2010. Stevioside and stevia-sweetener in food, application, stability and interaction with food ingredients. J. verb. lebensm. 5:225-229.

Kumari K L N W, Abeysinghe D C and Dharmadasa R M. 2016. Distribution of phytochemicals and bioactivity in different parts and leaf positions of *Stevia Rebaudiana* (Bertoni) Bertoni- a non-caloric, natural sweetener.World J. Agril. Res. 4(6):162-165.

Latham M. 2002. Macronutrientes: carbohidratos, proteínas y grasas. Nutriciónhumana en el mundo en desarrollo. 29: 99−107 Roma: FAO.

Lee C N, Wong K L, Liu J C, Chen Y J, Cheng J T and Chan P. 2001. Inhibitory effect of stevioside on calcium influx to produce antihypertension. Planta Med. 67:796–799.

Li G, Wang R, Quampah A J, Rong Z, Shi C and Wu J. 2011. Calibration and Prediction of Amino Acids in Stevia Leaf Powder Using Near Infrared Reflectance Spectroscopy. J. Agric. Food Chem. 59:13065–13071.

Liu J, Ong C P and Li S F Y. 1997. Subcritical fluid extraction of stevia sweeteners from *Stevia rebaudiana*. J. Chromatographic Sci. 35:446-450.

Liu J C, Kao P K, Chan P, Hsu Y H, Hou C C, Lien G S, Hsieh M H, Chen Y J and Cheng J T. 2003. Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. Pharmacology. 67(1):14-20.

Maira Segura-Campos, Enrique Barbosa-Martín, Ángel Matus-Basto, Diana Cabrera-Amaro, María Murguía-Olmedo, Yolanda Moguel-Ordoñez and David Betancur-Ancona. 2014. Comparison of Chemical and Functional properties of Stevia rebaudiana (Bertoni) Varieties Cultivated in Mexican Southeast. Am. J. Plant. Sci. 5:286-293

Makapugay H, Nanayakkara N and Kinghorn A. 1984.Improved high performance liquid chromatographic eparation of the *Stevia rebaudiana* sweet diterpene glycosides using linear gradient elution. J. Chromatography. 283: 390–395.

Mandal B and Madan S. 2013.Preliminary phytochemical screening and evaluation of free radical scavenging activity of *Stevia rebaudiana*Bertoni from different geographical sources. J. Pharm. Phyto. 2(1): 14-19

Marcinek K and Krejpcio Z. 2015.*Stevia rebaudiana* Bertoni–chemical composition and functional properties. Acta Sci. Pol. Technol. Aliment. 14(2): 145–152.

Mishra N. 2011. An Analysis of antidiabetic activity of *Stevia rebaudiana* extract on diabetic patient. Journal of Natr. Sci. Res. 1(3):1-9.

Mishra P, Singh R, Kumar U and Prakash V. 2010. Stevia rebaudiana-A magical sweetner. Global J. Biotech. Biochem. 5:62-74.

Mohammad M R, Mohammad U D, Sher M M, Habib A N and Iqbal A Q. 2007. *In vitro* clonal propagation and biochemical analysis of field established *Stevia rebaudiana* Bertoni. Pak. J. Bot. 39:2467–2474.

Mohanta T K, Patra J K, Rath S K, Pal D K and Thatoi H N. 2007.Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium.* L.f. Sci. Res. Essay. 2(11): 486-490.

Muanda F N, Soulimani R, Diop B and Dicko A. 2011. Study on chemical composition and biological activities of essential oil and extracts from *Stevia rebaudiana*Bertoni leaves. Food Sci. Technol. 44:1865–1872.

Nakamura Y, Sakiyama S and Takenaga K. 1995.Suppression of syntheses of high molecular weight on muscle tropomyosins in macrophages. Cell Motil Cytoskeleton. 31(4): 273−282.

Ou B, Hampsch-Woodill M and Prior R L. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluoresce in as the fluorescent probe. J. Agric. Food Chem. 49:4619–4626.

Pande S S, Gupta P. 2013. Plant tissue culture of *Stevia rebaudiana* (Bertoni): A review J. Pharmacog. And Phytotherapy. 5(1):26-33.

Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Flerlage N, Burillo J and Codina C. 2002. Comparison between the radical scavenging activities and antioxidant activity of six distilled and non distilled Mediterranean herbs and aromatic plants. J. Agric. Food Chem. 50:6882-6890.

Pasquel A, Meireles M A A, Marques M O M and Petenate A J. 2001.Extraction of *Stevia*glycosides with CO_2 + Ethanol, and CO_2 + water + ethanol.Brazil). Braz. J. Chem. Eng. 17(3):271-282.

Patil V, Ashwini K S, Reddy P C, Purushotham M G, Prasad T G. and Udaykumar M. 1996.*In vitro* multiplication of *Stevia rebaudiana.*Curr. Sci. 70(11):960

Preethi D, Sridhar T M and Naidu C V. 2011. Direct shoot organogenesis from leaf explants of *Stevia rebaudiana* (Bert.). J. phytol. 3(5):69-73.

Rajendran R and Sundararajan R. 2010. Preliminary phytochemical analysis and anti-bacterial activity of *Mimosa pudica*linn leaves. Int. J. Pharma and Bio. Sci. 1(2):1-8

Ranjan R K, Sathish-Kumar M, Seethalakshmi I and Rao M R K. 2013. Phytochemical analysis of leaves and roots of Mimosa pudica collected from Kalingavaram, Tamil Nadu. J. Chem and Pharma Res. 5(5):53-55

Rao A B, Prasad E, Roopa G, Sridhar S and Ravikumar Y V L. 2012. Simple extraction and membrane purification process in isolation of steviosides with improved organoleptic activity. Advances Biosci. and Biotechnol. 3:327-335

Rao S, Teesta V K, Avinish B, Krithika K and Bhat S. 2012. *In vitro* propagation of *Withania somnifera* and estimation of withanolides for neurological disorders. Journal of Pharmacog. 3(2):85-87.

Razak U N A A, Ong C B, Yu T S and Lau L K. 2014. *In vitro* micropropagation of *Stevia rebaudiana* Bertoni in Malaysia. Brazil. archives of boil. and technol. 57(1):23-28.

Rio D A, Obdululio B G, Casfillo J, Marin F G and Ortuno A. 1997.Uses and properties of citrus flavonoids. J. Agric Food Chem. 45:4505-4515.

Salah N, Miler N J, Pagange G, Tijburg L, Bolwell G P and Rice E. 1995. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. Arch. Biochem. Broph. 2:339-46.

Salim M U, Shaheed M H C, Mouoztaba M M K, Belal M U, Ahmed R and Baten M A. 2006. *In vitro* propagation of *Stevia rebaudiana* Bert in Bangladesh. Afr. J. Biotechnol. 5:1238- 1240.

Savita S, Sheela K, Sunanda S, Shankar A and Ramakrishna P. 2004. *Stevia rebaudiana* – A functional component for food industry. J. Human Ecol. 15:261–264.

Savita S M, Sheela K, Sunanda S, Shankar A G, Ramakrishna P and Sakey S. 2004. Health implications of *Stevia rebaudiana*. J. Hum. Eco. 15:191-194.

Schneider G and Wolfling J. 2004.Synthetic Cardenolidesand related compounds.Current Organic Chemistry.8(14).

Shah B A, Qazi G N and Taneja S C. 2009. Boswellic acids: a group of medicinally important compounds. Nat Prod Rep. 26:72-89.

Shakya A K. 2016. Medicinal plants: Future source of new drugs. International J. of Herbl. Medicine. 4(4):59-64

Shivanna N, Naika M, Khanum F and Kaul V K. 2013. Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. J. Diabetes & Its Complications. 27:103–113

Shukla S, Mehta A and Bajpai V K. 2013. Phytochemical Screening and Anthelmintic and Antifungal Activities of Leaf Extracts of *Stevia rebaudiana*. J. Biol. Active Products from Natur. 3(1):56-63

Shukla S, Mehta A, Bajpai V K and Shukla S. 2009. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food Chem. Toxicol. 47:2338–2343.

Shuvo M M A, Al–Mamun M, Chowdhury T, Absar N and Hasanuzzaman M. 2015. An assessment of major nutritional components and some secondary metabolites of *in vitro* propagated *Stevia Rebaudiana* (cultured in Bangladesh) plant leaves dry powder. Int. J. Applied Sci. and Biotechnol. 3(4):721-26.

Siddique A B, Rahman S M M, Hossain M A, Hossain M A and Rashid M A. 2014. Phytochemical screening and comparative antimicrobial potential of different extracts of *Stevia rebaudiana* Bertoni leaves. Asian Pac. J. Trop. Dis. 4(4):275–280.

Singh S, Garg V, Yadav D, Nadeem Beg M and Sharma N. 2012.*In vitro* antioxidative and antibacterial activities of various parts of *Stevia rebaudiana* (Bertoni). International J of Pharmacy and Pharmaceutical Science. 4(3):468-473.

Singh S D and Rao G P. 2005. *Stevia*: The herbal sugar of 21st century. Sugar Tech. 7:17-24.

Sivaram L and Mukundan U. 2003. *In vitro* Culture studies on *Stevia rebaudiana*. *In vitro* Cellular and Developmental Biology of Plant. 39(5):520-523.

Srivastava S, Chouhan D E S and Chawla P. 2016. Proximate and Phytochemical analysis of Stevia leaves powder. Int. Ayurvedic medical J. 4(8):2476-2480.

Stoyanova S, Genus J and Heideg E. 2011. The food additives inulin and stevioside counteract oxidative stress. Int. J. Food Sci. and Nutr. 62:207-214.

Szefer P and Nriagu J. 2007. Mineral components in foods (pp. 10). New York: CRC Press.

Tadhani M B, Jadeja R P and Rena S. 2006.Micropopagation of *Stevia rebaudiana* Bertoni using multiple shoot culture. J. Cell Tissue Res. 6:545-548.

Tadhani M B, Patelm V H and Subhashm R. 2007.*In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. J. Food Compost. Anal. 20:323–329.

Tadhani M B and Subhash R. 2006. Preliminary studies on *Stevia rebaudiana* leaves: proximal composition, mineral analysis and phytochemical screening. J. Med. Sci. 6(3): 321–326.

Tambe R, Inamake M R, Shelar P D, Kulkarni M S and Katekar S M. 2010. Isolation and analytical characterization of stevioside from leaves of *Stevia rebaudiana*Bert; (Asteraceae). Int. J. Res. Ayuveda and Pharm. 1(2):572-581.

Theis N and Lerdau M. 2003.The evolution of function in plant secondary metabolites. Int. J. Plant. Sci. 164:S93-S103.

Turkmen N, Sari F and Velioglu Y S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem. 93:713–718.

Uddin M S, Chowdhury M S H, Khan M M M H, Uddin M R, Ahmed R and Baten. 2006. *In vitro* propagation of *Stevia rebaudiana* Bert in Bangladesh. Afr. J. Biotechnol. 5(13):1238-1240.

Yadav A K, Singh S, Dhyani D and Ahuja P S. 2011.A review on the improvement of Stevia [*Stevia rebaudiana* (Bertoni)]. Can. J. Plant Sci. 91:127

Yang Y W, Hsing Y I and Chang W C. 1981. Clonal propagation of *Stevia rebaudiana* Bertonithrough axillary shoot proliferation. In vitro. Bot. Bull. Academia. 22:57-62.