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Steviosides and steviol glycosides extraction from stevia by utilizing various techniques

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Abstract
Stevia rebaudiana Bertoni consisting of steviosides and rebaudioside has wide range of applications worldwide as a sweetener and additive for the reduction of calories to produce low caloric food products. The stevia production has increased in most of the countries i.e., grown in California, Hawaii, Canada, Malaysia, Brazil, Korea, Thailand, Taiwan and China. The prominent Health improving potential of stevia and its adjunct therapy of many chronic diseases have been confirmed by several studies. Another unique attribute of stevia extract is thermostablity that makes it to use in baking and cooking of food products. There is still debate on extraction methods and toxicology of stevia. Hence, the purpose of this effort is to review the literature regarding nutritional value, toxicity and extraction techniques to get highly purified compounds (steviol glycosides and steviosides) and technological aspects of stevia.

Keywords: *Stevia rebaudiana,* sweeteners, extraction techniques

Introduction
Stevia contained almost 200 species of shrubs and herbs of Astracae family. *Stevia rebaudiana* with the ancient name *eupatorium rebaudianum bertoni* is the most important species from the Stevia genus (Yadav *et al*., 2011) [49]. It exhibited the greatest levels of sweetness among the other species i.e., *Stevia viscida, Stevia dianthoidea, Stevia serrata, Stevia salici- folia, Stevia rebaudiana, Stevia crenata, Stevia bertholdii, Stevia plummerae, Stevia phlebophylla, Stevia micrantha, Stevia lemmonii, Stevia eupatoria, Stevia enigmatica,* and *Stevia anisostemma* of Astracae family (Carakostas *et al*., 2008) [6]. The stevia also known as sweetleaf plant, is grown as annual plant in areas cold areas with temperature less than 0°C and grown as perennial plant in temperate areas as it requires little bit warm temperatures (15–30°C) and abundant rainfalls for optimal growth. The stevia plantation requires wet soil for proper growth and permeability. The height of stevia plant reaches up to 80 cm, it has oppositely arranged spatulate or lanceolate and oval leaves with diameter of 3-4 cm. The stem of stevia is woody with white or light violet five plated flowers (Chan *et al*., 2000) [7]. The chemical structure of Stevia rubaudiana is given in Figure 1.

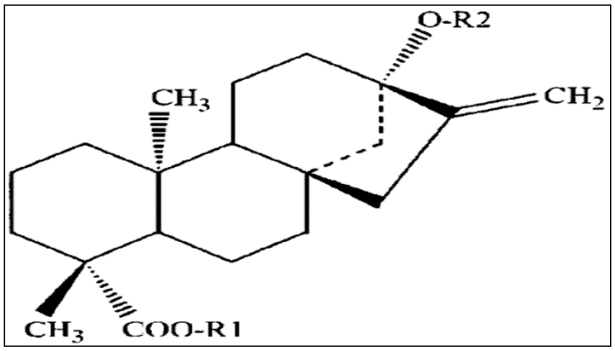

Fig 1: Basic Structure of Stevia Compound

A unique perennial bush native to South America, "*Stevia Rebaudiana Bertoni*" has an important factor to produce di-terpene glycosides (Rebaudioside A) which are low caloric sweeteners. It has a higher sweetness potential than other sweeteners including sucrose. Its sweetness intensity is 300-400 times more than standard sugar (Soejarto, 2001) [42]. Its extract is enriched with sweetening compounds such as steviol glycosides which are sweet in nature and have potential to act as an antimicrobial, antifungal and antioxidants agents (Marcinek & Krejpcio, 2016) [32]. It is confirmed that steviol glycosides improve the chemical veracity during the drying procedures and their extract can be used as an ingredient for functional foods. These functional foods will become the source of protein, minerals, carbohydrates and crude fiber, important for human nutrition (Chranioti *et al*., 2016) [9]. The review covered the history and nutritional composition of Stevia rebaudiana along with various extraction techniques of bioactive compounds from the Stevia rubaudiana.

History
Stevia Rebaudiana Bertoni is specie of genus "Stevia", family "*Asteraceae"* (sunflower family), first grown in the South America and aboriginal to the Amambay area in the northeast of Paraguay. It also available in the neighboring parts of Argentina and Brazil (Soejarto, 2001) [42]. Stevia is native to Rio Monday valley in Paraguay highlands, between 25-26° S latitude, it grow near areas of stream in sandy soils (Katayama *et al*., 1976) [22]. It is grown in California, Hawaii, Canada, Malaysia, Brazil, Korea, Thailand, Taiwan and China. The sweetness levels vary in relevancy to the presence of various bioactive compounds of stevia (Table 1).

Table 1: Structure of steviol glycosides on the bases of sweetness

Compound	R1	R2	Sweetener potency sucrose=1	Molecular weight
Dulcoside A	Glc(*β*)-	Rha(*α1-2*) Glc(*β*)	50-120	788.9
Rebaudioside A	Glc(*β*)-	Glc(*β1-2*) Glc(*β*)- Glc(*β1-3*)	250-450	967.0
Rebaudioside B	H-	Glc(*β1-2*) Glc(*β*)- Glc(*β1-3*)	300-350	804.9
Rebaudioside C	Glc(*β*)-	Rha(*α1-2*) Glc(*β*)- Glc(*β1-3*)	500-120	951.0
Rebaudioside D	Glc(*β1-2*) Glc(*β*)-	Glc(*β1-2*) Glc(*β*)- Glc(*β1-3*)	250-450	1129.2
Rebaudioside F	Glc(*β*)-	Xyl(*β1-2*) Glc(*β*)- Glc(*β1-3*)	130-250	937.0
Rubusoside	Glc(*β*)-	Glc(*β*)-	100-120	642.7
Stevioside	Glc(*β*)-	Glc(*β1-2*) Glc(*β*)-	150-300	804.9
Steviolbioside	H-	Glc(*β1-2*) Glc(*β*)-	100-125	642.7

Glc: D-gulucose, Rha: L-rhamnose, Xyl: D-xylose

The genus "*Stevia*" consists of almost 230 herbs and shrubs. *Rebaudiana* species included as 154th member of this genus. *S. rebaudiana* and *S. phlebophylla* are only two members of this genus which can develop sweetening compounds (Brandle & Telmer, 2007; Soejarto, 2001) [5] [42]. In 1887, Bertoni found this plant from the Mestizos and Paraguayan Indians. He studied the properties of stevia. Bertoni was the main stake holder who introduced this plant to the world (Lewis, 1992) [30]. He also classified *stevia* botanically and described it in more detail. It was pronounced as "*Eupatorium rebaudianum*", at the start, while in 1905 its name altered to *S. rebaudiana* Bertoni (Yadav *et al*., 2011) [49]. A brief history of stevia discovery leading to its current consumption particularly as sweetener and dietary supplement are presented in Table 2.

Currently, Stevia is well famous for its great sweetening components (diterpene glycoside) in dry leaf matter. Now, it is cultivated in many areas of the world containing Canada, Japan and some parts of Europe, Asia and China shares 95% production of stevia in the world (Kienle, 2014) [24] as shown in Figure 2.

Fig 2: Worldwide Regions for *S. rubaudiana cultivation*

Table 2: History leading to the origination and development of Stevia (Sativoside)

Year	Event	Reference
1887	First introduction to Europeans	Soejarto, 2001 [42]
1899	M.S Bertoni classified S. rebaudiana	Soejarto, 2001 [42]
	S. rebaudiana leaves are sweet, reported in a journal and get published	Gosling, 1901
1899	Initial chemical report on the sweet components of 'kaa he-e', noting that by 1899 the species was well known in Paraguay and used by herbalists to sweeten teas	Bertoni, 1905 [4]
1905	Name changed from "Eupatorium rebaudianum", to "*S. rebaudiana* Bertoni"	Soejarto, 2002 [42]
1905	Stevioside identification	Bertoni, 1918 [3]
1908	Realization of *stevioside* ('eupatorin') as a glycoside	Dieterich, 1908 [13]
1909	The sweet principle was first isolated	-
1931	stevioside a good source of steviolbioside	Wood *et al*., 1955 [48]
1931	Sweet glycoside was purified	Soejarto, 2001 [42]
1952	Diterpene glycoside was established as a Chemical structure of sweet glycoside	Soejarto, 2001 [42]
1961	Confirmation of structures of iso-steviols and steviol	Mosetigg *et al*., 1963
-	Firstly, Steviol was chemically synthesized	Cook *et al*., 1970 [10]
1965-70	Standardized extracts of purified Stevioside and *Stevia rebaudiana* and were available commercially in Japan	Soejarto, 2001 [42]
1970	Isolation and characterization of *rebaudioside A*	Kohda *et al.*, 1976
-	Minor sweet di terpene glycosides isolated in *S. rebaudiana* leaves	Kobayashi *et al.*, 1977 [27]
-	*S. rebaudiana* products get first approval in Brazil	Anonymous, 1980
1982	The Peoples Republic of China start large scale cultivation of *S. rebaudiana*	Kinghorn and sojareto, 1991 [26]
-	Mutagenic activity demonstration of metabolically activated steviol	Pezzuto *et al.*, 1985 [36]
1980-90	A famous herbal tea prepared with stevia leaves in the United States	Blumenthal, 1995 [4]
-	Stevioside get first approval of in South Korea	Anonymous, 1988
1991-95	Import ban of *S. rebaudiana* leaves into United States imposed by Federal Drug Administration (FDA 1991) and removed in 1995	Blumenthal, 1995[4]
1992	'Sugar transferred' (enzymatically modified) and rebaudioside A-enriched; *S. rebaudiana* extracts become more widely used in Japan in the early 1990s	Blumenthal, 1995[4]
1996	Toxicity test by the National Institute of Hygienic Sciences, Tokyo, Japan showing a lack of any carcinogenic effects.	Toyoda *et al.*, 1997 [47]
2008	The specifications for 7 steviol glycosides were established	Tada et al., 2013 [43]
2010	The sweetener stevia is approved as food additive in the form of steviol glycosides (E 960)	EFSA, 2010 [14]
2016	Patent to prepare pure STEVIOL GLYCOSIDESs	-
2016	Patent published for Stevia extracts	Markosyan *et al.*, 2016

Nutritional significance of Stevia

Natural sweetener sucrose, artificial sweeteners and others are considered as high in calorie contribution to diet. The nutritional composition and its mineral contents in various studies are given in table 3 and 4 accordingly. These can be metabolized completely by the body and have a potential to escalade towards overweight status, diabetes and other health issues as dental caries. A plant polysaccharide inulin type fructo-oligo saccharide found in roots and leaves of *S. rebaudiana* has some important functional properties related to prebiotics, dietary fiber, lipid metabolism and diabetes control. These polysaccharides are isolated from the leaves and roots of stevia plant, their yield was 0.46% and 4.65% respectively (Oliveira *et al*., 2011) [11]. This information provides a sound application of this extract as a nutritional supplement for consumers.

Analysis of stevia on a dry weight basis for determination of total energy has been reported by Gasmalla *et al*. (2014) [18] and it is estimated that total energy value by applying Atwater factor was 368.04 Kcal g^{-100}. Another study was conducted by Savita *et al*., (2004) [40] and they reported a 2.7 kcal g^{-1} energy value of dried stevia leaves. These results assured the low caloric value of stevia sweetener. It can be pronounced as a natural low caloric sweetener and its use as anti-diabetic, caries preventive will be very helpful to control and combat different health disorders. Moreover, studies also assured that it is more intense in sweetness than other available sweeteners.

It is evident that stevia contained great variety of essential amino acids (methionine, leucine, lysine, arginin, histidine, phenyl alanine, valine, therionine and isolucine) and non-essential amino acids (aspartate, serine, glutamic, proline, glycine, alanine, cystine and tyrosine) in their dried leaves (Abou-Arab *et al*., 2010) [1]. Vitamin analysis has been studied in stevia leaves as well as in callus (an isolated thickening of tissue) of stevia by Kim *et al*. (2011) [25]. He found that contents of vitamin B_2, vitamin C, and folic acid in the extracts of stevia leaves are significantly higher than those of the callus extracts and concluded that folic acid was present in abundant concentration followed by Vit. C, however in the extract of callus, Vit. C was present in higher concentrations followed by Vit. B.

Table 3: Chemical composition of dried Stevia leaves

Sr#	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)	Carbohydrates (%)	Reference
1.	7.46	4.39	12.44	5.26	8.06	69.85	Gasmalla *et al*. 2014 [18]
2.	ND	5.6	11.2	15	ND	53	Serio, 2010 [41]
3.	5.37	3.73	11.40	15.5	7.41	61.9	Abou-Arab *et al*., 2010 [1]
4.	7.7	2.7	12	ND	8.4	ND	Kaushik *et al*., 2010 [23]
5.	4.65	1.9	11.2	15.2	6.3	60.75	Goyal *et al*., 2010 [19]
6.	7	3	10	18	11	52	Mishra *et al*., 2010 [33]
7.	ND	4.34	20.4	ND	13.1	35.2	Tadhani *et al*., 2007 [44]
8.	7	2.5	9.8	18.5	10.5	52	Savita *et al*., 2004 [40]

ND = not determined

Its high ash content indicates that the stevia leaves are good source of inorganic minerals, and it will be beneficial for human health (Rai *et al*., 2013; Kaushik *et al*., 2010) [23].

Table 4: Stevia leaves (dried) minerals content mg 100 g^{-1}

Sr#	Ca (ppm)	Mg (ppm)	Cu (ppm)	Na (ppm)	Fe (ppm)	Ph (ppm)	Zn (ppm)	Mn (ppm)	Reference
1	17.7	3.26	0.73	14.93	5.89	ND	1.26	2.89	Abou-Arab *et al*., 2010 [1]
2	600	500	ND	ND	3.9	318	ND	ND	Serio, 2010 [41]
3	722	ND	ND	32.7	31.1	ND	ND	ND	Kaushik *et al*., 2010 [23]
4	544	349	ND	89.2	3.9	318	1.5	14.7	Goyal *et al*., 2010 [19]
5	464.4	349	ND	190	55.3	11.4	1.5	ND	Mishra *et al*., 2010 [33]
6	1550	ND	10.40	160	36.3	350	6.39	98.33	Tadhani *et al*., 2007 [44]

ND, not determined

Comparison of different methods for the extraction of steviol glycosides

The sweetening compounds of *Stevia rebaudiana* herb are steviol glycosides. The extraction of Steviol glycosides has an immense importance. There are many techniques present for the extraction of steviol glycosides (Table 5). Techniques utilized for extraction of stevioside are enlisted (Table 6).

Their efficiency is different from each other and utilized in different studies.

Table 5: List of extraction techniques for steviol glycosides steviosides

Sr.	Techniques	Reference
1	Solvent extraction	Pol *et al*., 2007; Thadani *et al*., 2006.
2	HPLC	Pol *et al*., 2007; Bernal *et al*., 2011 [2]
3	Chromatographic adsorption	Gasmalla *et al*., 2014 [18], Kolb *et al*., 2001 [28]
4	Ion exchange	Fuh & Chiang, 1990 [16]
5	Selective precipitation	Kaushik *et al*., 2010 **[23]**
6	Membrane processes	Zhang et al., 2000 [50], Fuh & Chiang., 1990 [16]
7	Gas chromatography	Bernal *et al*., 2011 [2]
8	Over-pressure TLC, densitometry, and capillary electrophoresis	Gardana *et al*., 2010 [17]
9	Supercritical fluids extraction	Erkucuk *et al*., 2009 [15], Choi *et al*., 2002 [8]
10	Enzymatic extraction	Puri *et al*., 2012 [38]; Kovylyaeva et al., 2007 [29]
11	Nuclear magnetic resonance	Bernal *et al*., 2011 [2]

HPLC (High performance liquid chromatography) is considered the simplest and most reliable method to determine the composition of *S. rebaudiana*. The efficiency of enzyme-assisted extraction of stevioside is more in yield than solvent extraction methods and it is considered as environment friendly (Puri *et al*., 2012) [38].

Table 6: Yield of steviosides by different extraction methods

Sr.	Name of method	Yield	Time (min)	Reference
1	Extraction with water			
	Water	4.7 [b]	50	Pól *et al*., 2007 [37]
	PHWE (Pressurized hot water extraction)	13.90 [a]	50	Teo *et al*., 2009 [46]; Tejo et al., 2013 [45]
	Cold-water extract of stevia leaves (4%)	0.61 % (wt/wt)	ND	Narayanan *et al*., 2014 [35]
	Erythritol and rebaudiana A (5.5%)	0.80 % (wt/wt)	ND	Narayanan *et al*., 2014 [35]
	Hot water extraction	7.60	ND	Abou-Arab, 1995 [4]
2	Extraction with organic solvent/ compound			
	Organic solvent	2.36 [a]	ND [d]	Choi *et al*., 2002 [8]
	Pressurized Fluid Extraction (PFE) Methanol	5.2 [b]	50	Pól *et al*., 2007 [37]; Rafiq et al., 2007 [39]
	Ethanol	48.66 [a]	100	Erkucuk *et al*., 2009 [15]
	Ethanolic extract	8 mg/ml	ND	Kaushik *et al.* 2010 [23]
	Na_2CO_3 precipitated	8 mg/ml	ND	Kaushik *et al.* 2010 [23]; Deshmukh *et al.* 2014 [12]
	CaO precipitated	8 mg/ml	ND	Kaushik *et al*., 2010 [23]
3	Super critical fluid extraction (SFE)			
	SFE (CO2-methanol–water, 80:16:4)	3.56 [a]	ND [d]	Choi *et al*., 2002 [8]
	SFE	36.66 [a]	100	Erkucuk *et al*., 2009 [15]
	Supercritical Fluid Extraction SFE (CO2-methanol, 80:20)	2.5[a]	ND [d]	Choi *et al*., 2002 [8]
4	Extraction through chromatography			
	Chromatographic Separation (Hexane, dicholroethane, ethyl acetate, methanol)	35.3 mg/600 g	1440	Pol *et al*., 2007, Mosettig et al., 1963 [34]
	High-speed counter-current chromatography (HSCCC)	26 [c]	270	Huang *et al*., 2010 [20]
5	Extraction through microwave			
	Microwave	8.64 [b]	1	Jaitak *et al*., 2009 [21]
	Microwave assisted Extraction	21.37 [a]	20	Teo *et al*., 2009 [46]
6	Extraction through ultrasound			

	Ultrasound	4.20 [b]	30	Jaitak *et al*., 2009 [21]
	Ultrasonic assisted extraction	43.62 [b]	32	Liu *et al*., 2010 [31]
7	Extraction through enzyme			
	Enzyme (Hemicellulase)	369 [c]	45	Puri *et al*., 2012 [38]
	Combination of three enzyme (Hemicellulase: Pectinase: Cellulase)	975 [c]	76	Puri *et al*., 2012 [38]

[a] mg/g, [b] %, [c] µg/mg· [d] Not determined

Conclusion

Stevia has unique nutritional values due to its bioactive compounds i.e., steviol glycosides and steviosides from its leaves. Stevia as a natural sweetener can be utilized for the management and treatment of various diseases such as diabetes. The various extraction methods can be used to obtain these compounds for further use in treatments of different ailments.

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