International Journal of Pharmacy and Pharmaceutical Science
www.pharmacyjournal.org
Online ISSN: 2664-7230; Print ISSN: 2664-7222
Received: 01-04-2019; Accepted: 02-05-2019; Published: 09-05-2019
Volume 1; Issue 2; 2019; Page No. 01-06

Stevia rebaudiana and its importance: A review

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DOI: https://doi.org/10.33545/26647222.2019.v1.i2a.7

Abstract
Stevia rebaudiana leaves are natural sweetener and it contain diterpene glycosides which taste sweet but with zero calories in food products. Stevia has been used by native Guarani Indians of South America for centuries as sweetener to counteract the bitter taste of various medicines and beverages. Diterpene glycosides produced by this plant are 100–300 times sweeter than sucrose and are now used as sweeteners to replace sugar in foods and beverages. They remain stable at wide range of temperature and pH conditions in food products during processing and offer no shelf life limitations. These glycosides do not induce glycemic response when ingested, making them suitable for diabetics and obese persons. India has huge demand potentials for this natural sweetener because of increasing diabetic and obese population. In this article effort was made to discuss briefly about plant profile and basic techniques of cultivation, harvesting, drying and extraction of steviol glycosides. Minerals content reported that the high content of K, Ca, Mg, Na of stevia were present in the leaf; whereas, Mn, Fe, Cu, Zn, and Cr were found as trace amount. The responsible compounds for the natural sweetness of stevia leaves include a diversity of diterpenoid glycosides derived from a steviol skeleton. These steviol glycosides also exhibit a low calorific value as well as promising therapeutic applications.

Keywords: Stevia rebaudiana; Steviol glycosides; Natural sweeteners; diterpene glycosides

Introduction
Stevia, a natural sweetener plant having medicinal and commercial importance and it is a perennial shrub belongs to the family (Asteraceae) Compositae. Stevia is native to Paraguay and Brazil and it is often referred to as ‘‘the sweet herb of Paraguay’’. It is also known as “honey yerba” and “honey leaf” and by some other variations of these names. The mature plant grows up to 65-centimetres (26 inches) to as tall as 180 cm (72 inches) when cultivated or growing naturally in fertile soil. It is a short-day plant and flowering from January to March in the southern hemisphere. It prefers a sandy soil, requiring a warm sunny position. The suitable natural climate is semi humid subtropical with temperature extremes from 21 to 43°C and average 24°C [1]. Moreover, S. rebaudiana is a perennial shrub of the Asteracease (Compositae) family native to certain regions of South America (Paraguay and Brazil). It is often known as “the sweet herb of Paraguay” [2]. The plant is also cultivated in China and Southeast Asia [3]. Stevia sweeteners, crude extract from leaves, are used to sweeten soft drinks, soy sauce, yogurt, and other foods in Japan, Korea and Brazil [4]. The dry extract from the leaves of stevia contains flavonoids, alkaloids, chlorophylls, xanthophylls, hydroxycynnamic acids (caffeic, chlorogenic, etc.), oligosaccharides, free sugars, amino acids, lipids and trace elements [5]. Stevia, the common name for the extract stevioside from the leaves of S. rebaudiana Bertoni, is 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 [6]. 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 [7]. The study of Mohammad *et al* [8] identified nine amino acids in stevia leaves, namely glutamic acid, aspartic acid, lysine, serine, isoleucine, alanine, proline, tyrosine and methionine. Abou-Arab *et al* [9] found still more amino acid in the stevia leaves. Altogether seventeen amino acids were determined and classified as essential and non-essential amino acids. Japanese have been using stevia and its products in cooked or baked goods, processed foods and beverages, fruit juices, tobacco products, pastries, chewing gum and sherbets [10].Stevioside and rebaudioside of Stevia are stable under wide range of temperatures and pH conditions in different food and pharmaceutical products [11, 12].They do not alter the flavour and taste of a food product in which they are used and are also non-fermentative. Stevia rebaudiana is commercially cultivated in China, Japan, Brazil, Canada, USA, UK, Spain, Belgium, Australia, South Korea, Thailand, Israel and Taiwan [13, 14]. China and Japan are the world’s major producers and exporters of diterpene glycosides. Japan has approved use of stevioside in many food products including cereals, teas, and soft drinks. In India, Steviahas been introduced in the last decade because of high demand potentials particularly considering the huge diabetic population. It has been successfully cultivated in many Indian states like Rajasthan, Maharashtra, Punjab, Kerala and Orissa. High demands for natural sweeteners as compared to artificial ones have driven the farmers in India toward large-scale Stevia cultivation [15].In this article we discussed about some important aspects of Stevia cultivation, production and utilization of zero calorie natural sweeteners.

Plant Profile
Stevia rebaudiana is a subtropical perennial herb, belonging to family Asteraceae. It has annual, subligneous, more or less

pubescent stems with extensive, fibrous and filiform root system [1]. The cultivated Stevia plant grows vigorously giving branched bushy shrub like appearance [16]. It grows up to 60-70 cm in height and bears sessile, oppositely arranged lanceolate to oblancoelate leaves with blunt-tipped lamina having serrate margin from the middle to the tip. The upper surface of the leaf lamina is slightly glandular. Plant bears small (10-15 mm) white colour pentamerous flowers in capitulum surrounded by green colour involucral bracts. The capitula are arranged in irregular or sympodial cymes. Seed of Stevia is a five-ribbed spindle-shaped achene with feathery pappus. Plant is diploid and has 11 chromosome pairs [17].

Fig 1: Stevia rebaudiana Plant (google.co.in)

Plant Varieties

There are about 90 varieties of stevia rebaudiana developed all around the world. Basically all these varieties have been developed for different climate requirements, many times these varieties perform strangely in different climate conditions. At the end of the day, just like sugarcane, it is the stevioside and rebaudioside content in the Stevia leaves that determine the price and marketability of Stevia leaves. In many causes in south India it was observes that stevioside content was as low as 3.5%, which was below the minimum market requirement of 9%. Hence it becomes imperative that the grower selects proper varieties with adequate guarantees from the planting material suppliers about minimum assured stevioside and rebaudioside contents.

Cultivation

In 1964 this crop was cultivated commercially for the first time in Paraguay and later on it has been introduced in a number of countries [18]. Stevia rebaudiana is now successfully growing under different cultivation conditions and climatic locations of the world. Seeds produced by Stevia plants remain viable for a limited period and have very low germination rate because of their small size. Being highly heterozygous species, there is also variation in plants raised from seeds. They do not produce true to type plant and constant re-selection for type is required in mother-seed plots [19]. Stevia is mainly propagated vegetatively by stem cuttings although it is a labour intensive procedure. Careful selection of plant is required to get more productivity and profitability. Cutting should be obtained from a plant variety which have reduced tendency to flower. Leaves of plant variety selected for propagation should be high in Rebaudioside-A and low in Stevioside associated with bitter after taste. Stem cuttings of selected plant variety should be 3-4 inches long with atleast one or two buds arising from leaf axils. Rooting can be enhanced by using rooting hormones. Treatment of cuttings with synthetic auxins was found beneficial for root formation by Steviagrowers. Stevia prefers a well-drained fertile sandy loam or loam soil high in organic matter. It prefers lighter acidic to neutral (pH 6-7) soil for better growth. It requires a consistent supply of water but excessive irrigation in water logging soils can cause stem rot disease. Stevia requires partial shade during very hot and long summer days. Photoperiod is more critical than intensity of light for Stevia cultivation. Long spring and summer days favor leaf growth and short days trigger blossoming in Stevia plant. Temperature in the range of 24 to 35 degrees with appropriate soil moisture is required during first two weeks to obtain plantlets from stem cuttings. Initial growth from cuttings is extremely slow and requires good nursery hygiene to prevent disease infection. Stevia plants produce two to three shoots depending on the number of buds available on stem segments. These shoots then produce multiple shoots, which is essential for production of good number of leaves for harvest. 20 to 35oC temperature are for proliferation of shoots from cuttings after field transfer. High temperature and water stress is unfavorable for vegetative growth as it induces flowering during the expected growing season. Fertilizer requirement for Stevia is moderate and varies according to the environment and soil type. Stevia plants respond well to fertilizers. Plant prefers low levels of nitrogen but high phosphorus and potassium. Under average climatic conditions and soil type 70 kg Nitrogen, 35 kg Phosphorus and 45 kg potassium per hectare is recommended. The distance between plants should be 20-25 cm. This would give a plant population of around 28 to 30 thousand per acre. Katayama *et al* [20] have tried planting densities ranging from 40000 to 400000 plants per hectare in experiments conducted at Japan. They found that leaf yield increases with increasing planting density up to 83,000 and 111,000 plants per hectare. For Stevia cultivation, land should be initially harrowed and then ploughed to get fairly smooth and firm planting surface. Fields should be divided into plots of convenient size for proper drainage and irrigation Formation of raised beds is the most economical way to grow Stevia plants as it saves water and avoid the damages caused due to water logging. The raised beds should be of 15 cm in height and 60 cm in width.

Irrigation

Stevia requires ample supply of good water all year around. As the plant cannot tolerate drought, frequent irrigation is required Micro sprinklers are the best method of irrigation would not supply the required amount of water at the right time. So through micro sprinklers, the water can be sprinklers, the water can be sprinkled once in a day in winter and two to four times in a day in summer depending upon the heat and relative humidity in the air. Watering frequency should be scheduled so that the plants do no wilt for want of water.

Harvesting

Stevia is harvested when plants are mature and blooms have just begun to form, generally in late summer. Sweetness is intensified by cooler temperatures and short days; however, sugar levels decline after flowering. Large-scale commercial growers use a specially designed harvester that cuts the crop at ground level, but small plots are often harvested by hand. It is possible that a rotary

mower with a bag attachment could be adapted for plots too large for hand-harvesting, but be sure to use a separate, designated unit to prevent contamination from other crops. Plants are dried with low heat and good air circulation for 24 to 48 hours. An alfalfa or grain dryer could be used or plants can be dried in the sun. Dried plants are then threshed to separate the sweet leaves from the bitter stems. Dried leaves can be stored for long periods in air-tight containers or plastic bags. Canadian growers store the dried leaves in sealed, plastic-lined boxes prior to processing. Home processing generally involves either crushing the ground leaves or making a crude extract using a water extraction process. Commercial processing to isolate pure rebaudioside-A for the various sugar substitutes on the market involves a much more complicated procedure. Some of these extraction processes are patented.

Drying

Drying is the important activity in post-harvest handling of Stevia. Freshly harvested leaves of Stevia contain high moisture content and deteriorate if not dried properly. Drying of leaves should be completed immediately after harvesting by placing leaves on a screen or net. Sun drying is the most preferred method as Stevia leaves can be quickly dried in moderately warm conditions in about 12 hours. Proper aeration with low density of loading is required for quick drying in full sun. It can also be dried in a simple dryer by passing hot air just above room temperature. Samsudin and Aziz [21] observed that quality of leaves dried at 50°C temperatures in hot air dryer for 6 hours was better in terms of colour, sweetness and nutrient content. Dried leaves with 3-5% moisture content should be packed in air tight container and stored in cool and dry place.

Extraction of Steviol Glycosides

Extraction of steviol glycosides from leaves involve many conventional processes and long purification procedures. It is somewhat similar to the extraction process used in sugar mill. Extraction of glycosides from leaves of Stevia involves aqueous or solvent extraction, ion exchange purification, precipitation, filtration, crystallization and drying [22]. Extraction protocol involves dissolving leaves in hot water or alcohols. Some authors have treated leaves with non polar solvents such as chloroform to remove oils, lipids, chlorophyll and other non-polar substances. Afandi *et al* [23] revealed that methanol is the best solvent for the extraction of Rebaudioside-A from Stevia leaves in terms of component yield. In their study ethanol and aqueous acetone were also found suitable to extract Rebaudioside-A, but yield was less compared to methanol. Extract was then clarified by precipitation with salt or alkaline solutions, concentrated and redissolved in methanol for crystallization of the glycosides. The common steps involved in the extraction procedure as described by Rank and midmore [19] are: soaking the leaves in warm water to dissolve the glycosides, precipitation and filtration of the resultant solution, concentration by evaporation, ion exchange purification, spray drying and crystallization to produce white powder/crystals. Rao *et al* [24] used ultra and nano-filtration membrane to developed simple, inexpensive and eco-friendly process for isolation of steviol glycosides with improved taste profile of the final product. Most of the commercial processing of Stevia leaves for the extraction of steviol glycosides is mainly concentrated in China and Japan where factories are located near cultivated areas.

Safety Aspect and Uses

Many biological and toxicological investigations were carried out on steviol compounds of Stevia in last 50 years. European Commission's Scientific Committee on Foods (SCF) evaluated safety related issues of this natural sweetener in 1985 and 1999 and raised questions about lack of acceptable purity specifications. In 2004, Joint FAO/WHO Expert Committee on Food Additives (JECFA) established tentative purity specifications which were later made permanent. JECFA have established Acceptable Daily Intake (ADI) of 4 mg/kg bodyweight/day for purified steviol glycosides in 2008 and validated its use as a sweetener in food and beverages. European Food Safety Authority (EFSA) in 2010 also gave ADI in consistent with that of JECFA and concluded that diterpene glycosides of Stevia are safe. Steviol glycosides (E960) have recently been added to the European Union (EU) list of permitted sweeteners. Following approvals by the United States Food and Drug Administration (USFDA) and the European Union (EU), Food Safety and Standards Authority of India (FSSAI) has also recently recommended use of Stevia as sweetener in selected food products with ADI limits. Large numbers of studies on these glycosides are available in scientific literature related to safety aspects and most of them supported the validation of regulatory agencies around the world. World Health Organization has estimated that Stevia would replace about 20% of the sugar market. Stevia products and extracts are now used in a variety of food products. The steviol glycosides obtained from Stevia leaves are used as an alternative to sugars in variety of foods and beverages. Stevia sweeteners can also be used in combination with other sweeteners (e.g., sugar, fructose etc) as they act as flavour enhancer in food products. They remain stable in food processes such as extrusion, pasteurization, baking, canning etc with no shelf life limitations. Steviol glycoside reduces dental problems as they are free of calories and mild on the teeth. They are especially suitable for diabetics as they do not affect blood sugar levels. FSSAI has recently approved use of steviol glycoside as a non-calorific sweetener in following 11 food items:

1. Dairy based flavored drinks
2. Dairy based desserts
3. Yoghurt
4. Fruit nectars
5. Non-carbonated water based beverages
6. Ice Lollies/Edible Ice
7. Jams, jellies and marmalades
8. Ready to eat cereals
9. Carbonated water
10. Soft Drink Concentrate
11. Chewing gum

Production Consideration

Greenhouse-Grown Transplants

Stevia transplants can be produced under similar greenhouse conditions as common flower and vegetable transplants. Plants

Can be produced in multi-pack containers or in individual 4- or 6-inch containers. Plants can be produced from seed or cuttings, and small greenhouse producers should consider purchasing plugs from wholesale plug specialists for finishing in larger containers. Vegetatively produced propagules from reliable sources are preferred to ensure production of plants with consistent sugar (glucoside) profiles.

Field-Grown

Stevia prefers well-drained soil rich in organic matter. Do not plant in field sites subject to flooding or puddling, or in saline soils. While plants are somewhat drought-tolerant, a consistent source of moisture should be supplied via trickle irrigation. Most sources recommend frequent, shallow irrigations because stevia's feeder roots tend to be produced near the soil surface. Research in Ontario has shown that stevia has low nutrient requirements, and excess nitrogen can result in profuse plant growth with poor flavor. Stevia is planted in spring after all danger of frost has passed. Vegetative propagation via tissue culture or rooted cuttings is preferred for obtaining the most consistent results in terms of plant characteristics, but may be cost prohibitive compared to seeds. Stevia can vary in sweetness from plant to plant; therefore, cuttings should be taken only from sources known to have high concentrations of sugar. Seeds can germinate poorly, so direct-seeding to fields is not recommended; however, plants can be propagated from seed in the greenhouse. Plugs may be purchased from herb wholesalers, or growers could produce their own transplants from high-quality seed. Research has shown that only the black or dark seeds are viable, while the clear to tan ones are often sterile. Several seeds should be placed in each plug cell, and then thinned to one seedling per cell once seeds have germinated. Time from seed-to-transplant is approximately seven to eight weeks. An estimated 20,000 to 40,000 plants will be needed for a 1-acre planting.

Pest Management

Field-grown stevia is not known to have serious insect pest problems and is often reported as exhibiting insect-repellant qualities. Similarly, documented disease problems are few and seemingly insignificant. Septoria diseases have caused damage on overly mature plants. Aphids, thrips and white flies can become a serious problem on stevia in greenhouses, which could significantly impact transplant production. Research in Kansas indicated that rabbit and deer feeding was not a problem in their stevia plots. The lack of herbicides registered for use with stevia means growers will need to control weeds with alternative methods, such as mechanical cultivation and by hand.

Labor requirements

Potential labor needs for field-produced stevia will be highly variable depending on market channel and product marketed. Hand planting, harvest, and packing products for retail would make stevia a labor-intensive crop, similar to culinary herb crops that are dried and packaged. Stevia grown for bedding plant production would have similar labor requirements as other annuals that are planted, transplanted, and harvested for sale as bedding plants.

Economic considerations

Initial investments for greenhouse-produced transplants are consistent with other greenhouse-produced bedding plants. When produced as an additional transplant species within an existing product mix, average production costs are approximately $1.50 per square foot of greenhouse space. Initial investments for field-produced plants include land preparation, purchase of seed or transplants, and installation of an irrigation system. Additional costs for a minor niche crop like stevia may include product and market development, advertising and consumer education Wholesale and retail prices for stevia are highly variable, and this price instability indicates that stevia is still an emerging and experimental new crop. It is likely that stevia, when grown in a 1-acre mix of other edibles and bedding plants, could help generate returns to land, labor, and management similar to culinary herb production. Producers should never plant a new niche crop without a well-defined plan for marketing and selling their crop [25, 26].

Chemical Components

Stevia is a natural sweetener plant. The leaves of stevia are the source of glycosides. Even more, stevia contains a high percentage of phenols, flavonoids and antioxidant activity [27]. The two main glycosides are Stevioside (St), traditionally 5-10% of the dry weight of the leaves, and Rebaudioside A (R-A), being 2-4%; these are the sweetest compounds. There are also other related compounds including Rebaudioside C (1-2%) and Dulcoside A & C, as well as minor glycosides, including flavonoid glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils [28]. Leaves of stevia contain around 10 sweetening glycosides of which stevioside (3-10%), rebaudioside-A (13%), and rebaudioside-B, C, D are important [29]. Eight phytochemical properties of stevia glucosides were discovered, viz. dulcosides A, rebaudiosides A-E, steviobioside and stevioside [30]. In addition, the triterpenes amyrin acetate and 3 esters of lupeol and the sterols like stigma sterol, sitosterol and campesterol can also be extracted from the leaves [31]. Noncaloric, natural sweeteners which are safe for diabetics might receive greater focus in future. There is greater interest in this plant now as a natural alternative to artificial sugar [32].

Future Aspect of Sweetener Plant

The leaves of this splendid plant are 30 times sweeter than sugar; with zero calories where as pure extract is 300 times sweeter than sugar. This sweet-honey -leaf herb is likely to become the major source of high potency sweetener for the growing natural food market, in the years to come. Stevia finds its use as a natural sweetener, replacing the chemical sweeteners and even table sugar; the sweetness in leaf is due to the presence of an intensive-sweetening agent called stevioside and the leaf by itself is about 20 to 30 times sweeter than sugar. The leaf has stevioside of 10-12% on dry weight basis. Stevia is a new promising renewable raw material for the food market. The market potential for this natural sweetener is steel untapped. It is estimated that about 30 million Indians are presently suffering from diabetes and it is estimated that by 2025 India's contribution to the diabetic global

Population would be a whopping 89 million. With such a huge share of the population being diabetic, the new ventures in the food industry are focused entirely on them. The soft drink manufactures in India are yet to exploit the sweetness of this herb by its addition in their product. Though many soft drinks are introduced in the market with the prefix 'dia' connoting that it meant for the diabetics, the usage of stevia in such products would fetch a greater demand than for the one with artificial sweeteners. The beverage industries like tea and coffee manufacturers have just started introducing new products for the diabetic patients, realizing the major share held by them in the consumer market. As tea and coffee has been the non -replaceable and the best beverage for every Indian, this sector has enormous potential to come up when the natural sweetener Stevia is used in their products. All those 'dia' prefixed products in the market at present are sweetened with artificial sweeteners that is, of course, equally sweet, but with undetectable side effects in due course. Stevia is a fully plant -based, natural sweetener can be used to replace the artificial sweeteners completely. As stevia leaf powder with no processing in highly safe to use, calorie-free and moreover around 20-30 times sweeter than cane-sugar, it can replace cane-sugar too. The process of manufacturing Stevia leaf powder is quite simple, when compared to the tedious steps involved in cane sugar manufacture.

Conclusion

Stevia (Stevia rebaudiana Bertoni) is gaining significant popularity in different parts of the world. Climatic factors and agronomical practices have impacts on quantitative and qualitative characteristics of stevia plants. Time of harvesting depends on land type, variety and growing season. Leaves of stevia gain optimum yield and quality stevioside just before flowering. Stevia is mainly used as a sweetener and flavor enhancer in the food and beverage industry. The compound obtained from stevia is considered to be the best alternative source of sweeteners for diabetes patients. Moreover, the unique selling points of stevia sweetener are very strong in Bangladesh due to the prevalence of diabetes and other metabolic diseases including obesity. However, further studies are required to identify standard cultivation areas and technology to understand the relationships that are responsible for quality stevia production. The requirement for higher influence sweeteners is anticipated to increase Worldwide. The increasing in the number of diabetic patients and health conscious individuals would push forward the need for alternatives to sugar. Stevia is a potential alternative source for replacing artificial sweeteners like saccharin, aspartame, asulfam, etc. Unlike many low calorie sweeteners, Stevioside is stable at. High temperature and over a pH range of 3 -9. Steps need to be properly aligned to exploit the natural sweetness of Stevia. Food industrialists have to start launching new products utilizing Stevia. This would obviously provoke the need to grow more and finally result in more area under Stevia cultivation. In India several important and necessary steps have to be taken up for its propagation. Development of seedlings suitable to India would of the first requirement. A crop production system, providing information on optimized crop inputs, weed and disease control, harvesting and handling methods would have to be detailed out. Awareness has to be created about the natural herb and the products manufactured out of it by the industries. It's time to streamline necessary forces to have access to Stevia. Initiative needs to be undertaken to promote this natural sweetener and create product awareness.

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