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STEVIA REBAUDIANA: ITS EXTRACTION, COMPOSITION AND MEDICINAL APPLICATIONS

Bina Rani[1], Raaz Maheshwari[2], A K Chauhan[3], Pooja Sharma[4], Surabhi Sharma[4]

1. Department of Environmental Engineering and Environmental Chemistry, PCE, Sitapura, Jaipur–302 020
2. Department of Chemistry, SBRMGC, Nagaur, Rajasthan, India
3. Department of Chemistry, SKGC, Sikar, Rajasthan, India
4. Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

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For Correspondence:

Raaz Maheshwari

Department of Chemistry, SBRMGC, Nagaur, Rajasthan, India

E-mail:

binaraj_2005@rediffmail.com

ABSTRACT

India is the largest consumer of sugar in the world and the country has a fairly high population of diabetic people (about 15%) in the age group of 25-45, which in increasing at an alarming pace. Due to sedentary lifestyles that we all tend to lead these days, the incident of obesity and diabetic conditions is constantly increasing dramatically. This single factor we have come to understand would greatly contribute to increasing the number of diabetic people and related maladies. Stevia extract is considered to be free of calories, carbohydrates, fats and cholesterol. So, it can be safely used by the diabetic patients. Therefore, the cultivation of stevia with modern agro techniques is gaining importance in India and it has been selected as an alternative crop in many states of India with high return support to the farmers.

INTRODUCTION

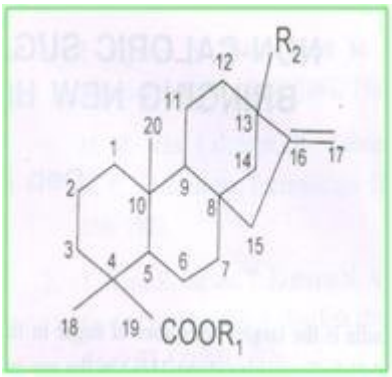
Stevia rebaudiana is relatively a new crop in the Indian Sub-continent and gaining very high popularity amongst all types of sweetener users as the most ideal substitute for sugar. In this changing lifestyles and more consciousness of health, worldwide sugar consumption is going down and is getting replaced by low calorie sweeteners. Many of these sweetners are complex chemicals or many a time naturals as well. Stevia is 30 times sweeter than sugar. At present, stevia accounts for just one per cent of the global sugar-substitute market, though its value in the US market is expected to grow to $2,000 million by the end of 2011, from $21 million in 2008, according to a prediction by research group Mintel. The Indian market can get boost from the government. The average sugar yield from a 0.4 ha sugar cane field is four tonnes; average yield of stevia extract that equals sweetness of sugar is over 60 tonnes from 0.4 ha. The market of stevia in India is limited because there are no processing units. The government run Institute of Himalayan Bioresurce Technology at Palampur, HP is planning to set up the country's first patented eco-friendly stevia processing unit near Delhi (The unit's capacity won't be high – 3000 leaves a day.) artificial sweeteners are mired in controversy, some of them were found to be carcinogenic. A study by the Centre of Advanced Studies (CAS) in the university of Kolkata found acesulfame potassium and saccharine could damage the DNA, Sacchrine is also associated with memory loss. Plant based sweeteners are safer. In accordance with the study carried out on stevia by the privately run Institute of Bioscience and Biotechnology Research and Development (IBBRD) at Kochi in Kerala. "All organs were found to be normal even at high dosage of 2,000 mg stevioside per bodyweight," stated the director and lead author of the study published in *Toxicology International* in June, 2010. It is a natural sweetener and calorie free. But soft drink makers don't prefer it in beverages. The reason is the Food Safety and Standards Authority of India (FSSAI) is focused on artificial sweeteners and does not recognize other sugar substitutes.

BOTANICAL FEATURES

Stevia is a member of the sunflower family (*Asteraceae*). It is native to the valley of the Rio Monday in highlands of Paraguay, between 25 and 26 degrees south latitude, where it grows in sandy soils near streams, Katayama et. al. (1976). It is a small shrubby perennial growing up to 65 cm tall, with sessile, oppositely arranged lance lanceolate to oblanceolate leaves, serrared above the middle. The flowers are small (7-15 mm), white and arranged in an irregular cyme. The seed is an achene with a feathery pappus, Robinson (1930).

CHEMICAL COMPOSITION OF STEVIA SWEETENERS

Although interest in the chemistry of the sweetening principles dates from very early in the century, significant progress towards chemical characterization of stevioside, secrecy of stevia was established, Bridal and Lavieille (1931). From the extensive research it was revealed that the sweetening principle in stevia is due to natural sweet active components present in the leaves of *Stevia rebaudiana* that is stevioside and rebaudiosides A, B, C and D; dulcoside A; and steviolbioside. Stevioside has a slighter bitter aftertaste and provides 250 to 300x the sweetness of the sugar. It is at 200°C (392°F), but it is not fermentable and does not act in browning reactions. Treatment of with the digestive juice of a snail yielded three moles of glucose and one mole of steviol, while acid hydrolysis gave isosteviol, Bridel and Lavieille (1931). Isosteviol was also obtained when steviol was heated in dilute sulphuric acid. Subsequent studies have lead to the isolation of seven other sweet glycosides of steviol. Typical proportions, on a dry weight basis four the four major glycosides found in the leaves of wild stevia plants are 0,3% dulcoside, 0.6% rebaudioside C, 3.8% rebauside A and 9.1% stevioside.



COMPOUNDS	R1	R2
Stevioside	β – glc	β – glc$^{2}_{\beta}$ – ^{1}glc
Rebaudioside A	β – glc	β – glc$^{2}_{3}$ < $^{\beta_1 glc}_{\beta_1 glc}$
Rebaudioside C	β – glc	β – glc$^{2}_{3}$ < $^{\alpha_1 rham}_{\beta_1 glc}$
Rebaudioside D	β – glc$^{2}_{\beta}$ – ^{1}glc	β – glc$^{2}_{3}$ < $^{\beta_1 glc}_{\beta_1 glc}$

Fig.1 Structure of stevioside

Structure of steviol, isosteviol and stevioside: The structure, stereochemistry and absolute configuration of steviol and isosteviol were established through a series of chemical reactions and correations over 20 years after pioneering work of Bridel and Lavielle. Chemically, stevioside is 10-O-glucopyranousyl-13-O-glucopyransyl (1,2)-glucopyransyl stevia [MW: 804.2]. Structure of these and oother diterpenes and diterpene glucosides are presented in Fig. 1. Concurrent Sweet components in Stevia Extract studies on the parent glucoside indicted that on the parent glycoside indicated that on D-glucopyranose residue, hydrolysed under alkaline conditions yielding steviolbioside, was attached to a carboxyl group while the other two were components of sophorosyl group bound to tha aglycone through a α-glycosidic linkage. Support for the proposed stereochemistry was achieved by the synthetic transformation of steviol into stevioside, Ogawa et. al. (1980). In 1993, spetrocopic data concerning stevioside and steviolbioside were published, Van et. al. (1993).

HISTORICAL BACKGROUND OF ITS USE

The guarani Indians had known for centuries about the unique advantages of *kaa-he-he* (a native term which translates as "sweet herb") long before the invaders from Old World were lured by the treasures of the New. These native people knew the leaves of the wild stevia shrub (a perennial indigenous to the Amambay Mountain region) to have a sweetening power unlike anything else; they commonly used the leaves of bitter mate (a tea-like beverages) and medicinal potions, or simply chewed them for their sweet taste. The widespread native use of stevia was chronicle by the Spaniards in historical documents preserved in the Paraguayan National Archives (PNA) in Asuncion. Historians noted that indigenous people had been sweetening herbal teas with stevia leaves "since ancient times." In due course, it was introduced to settlers. By the 1800s daily stevia consumption had become well entrenched throughout the region-not in Paraguay, but also neighbouring Brazil and Argentina.

Like the discovery of America itself, however, credit for stevia's "discovery" goes to an Italian. In this case, the explorer was a botanist whose initial unfamiliarity with the region caused him to believe that he had stumbled onto a "little known" plant. Dr Moises Santiago Bertoni, Director of the College of Agriculture in Asuncion, first learned of what he described as "this very strange plant" from Indian guides while exploring Paraguay's eastern forests in 1887. This area was not the herb's native "growing ground" Consequently, Betoni, by his own account was initially

"unable to find it." It was 12 years before he was presented with tangible evidence – a packet of stevia fragments and broken leaves received from a friend who had receives them from the mate plantations in the Northeast. He subsequently announced discovery of the "new species" in botanical journal published in Asuncion.

In 1970, the stevioside and stevia products were introduced in Japan and the products quickly caught on. By 1988 they reportedly represented ~ 41% of the market share of potently sweet substances consumed in Japan. In addion to widespread use as a tabletop sweetener, like the packets of saccharine ("Sweet-n-Low") and aspartame ("Equal") commonly found in the US, stevia was also used y the Japanese to sweeten a variety of products, including ie cream, bread, candies, pickles, seafood, vegetables and soft drinks.

AWARENESS AROUND THE WORLD

Stevia has a long history of safe usage with more than 150 safety and Government approvals in many countries across the world for centuries, the native Gurani Indians of South America have been safely using stevia. They have been using stevia leaves to sweeten and enhance the taste of their food and beverages. For over 30 years, stevia has been extensively used in countries like Japan, China and Korea as a cane sugar substitute and also to replace low calorie artificial sweeteners. In so many years of its use, no harmful effects have been reported by the stevia users. Today, stevia is commercially grown and used in countries including Japan, China, USA, Canada, and Australia, Ministry of Health and Welfare, Ministry of Healh Inspection Authority, US Food and Drug Administration (FDA), Australian Register of Therapeutic Goods (ARTG) and other fifteen countries across the world (including India) approve it which support the safety of stevia. In India, it was simultaneously introduced in Pune as well as Bangalaru and the demant for high quality stevia leaves is increasing dramatically day by day. The cultivation of stevia with modern agro-techniques is gaining importance in India and it has been selected as an alternative crop in many states of India with high return support to the farmers. Looking at the nature and agro climatic conditions like climate, soil moisture, and pH, the Government of India has proposed one each processing units in Western and Southern States. At the same time, its potential is under active consideration by Indian Ayurvedic medicine majors.this new crop would give Indian farmers a choice of a profitable and healthy crop to satisfy palates of eternally sweet toothed Indians.

COMMERCIAL EXTRACTION OF STEVIOL GLYCOSIDES

Most of commercial processing of stevia leaves occurs in Japan and there are dozen of patents describing method for the extraction of steviol glycosides. The extraction is based on the solvent (Haga et. al., 1976), solvent plus a decolorizing agent (Ogawa, 1980), adsorption chromatography (Itgaki and Ito, 1979) or ion exchange (Uneshi et. al., 1977) and selective precipitation of individual glycosides (Matsushita and Kitahara, 1981). The most favoured extraction process (Phillips, 1989) involve four steps: aqueous or solvent extraction, ion exchange, precipitation or coagulation with filteratio, then crystallization and drying. Recently, new methods based on ultrafilteration have been developed, Tan and Ueki et. al., 1994.

MEDICINAL APPLICATIONS OF STEVIA

Stevia extract are considered to have few calories, carbohydrates, fats and cholesterol. So , it can be safely used by diabetic persons.Stevia also acts as flavouring agent. It brings out true flavours in cereals, breads, icecreams, tooth paste and mouth wash. It is used to aid digestion, losing weight and stimulating appetite. The sweetening effect of these compounds is purely by taste, they are undigested and the body absorbs no part of the chemicals, they are , therefore of no nutritional value.one-fourth teaspoonful of dried leaves (finely ground) is claimed to have a sweetening value equal to one 1 cup of sugar. Stevia is a completely safe specific herb for diabetes a hypoglycaemia, a flavour enhancer, it contains a variety of constituents, besides the stevioides and rebaudiosides the nutrients and good deal of sterols, triterpenes, flavonoids, tannins. Stevia also contains an extremely rich volatile oil comprising rich proportions of sesquiterpenes. So far these constituents probably have some impact on human physiology and may help explain some of the reported beneficial therapeutic uses of stevia.

Blood-Sugar Normalizer: it is probably the of steviosides themselves that has produced dozan of empirical and semi-controlled reports of antihypoglycemic action. In different places of the world, it is believed that stevia is helpful for hypoglycaemia and diabetes because it nourishes the pancreas and thereby helps to restore normal pancreatic function. In semi-controlled clinical reports, one also encounters this action. Similar trends have been reported in humans and experimental animals by other workers. In Brazil, stevia casules are officially approved for sale for the treatment of diabetes. Stevia shows a normalizing tendency to blood sugar i.e. it brings high blood sugar down, and has no effect on persons with normal blood sugar levels.

Cardiovascular Action: A good deal of experimental work has been done on the effects of stevia and stevioside on cardiovascular functioning in human and animals. Some of this work was simply looking for possible toxicity, while some was investigating possible therapeutic action. In neither case significant changes have been found, only thing observed was a slight lowering of arterial blood pressure at low and normal doses. The most curious finding is a dose dependent action on heart beat, with a slight increase appearing at lower doses, changing to a mild decrease at higher doses. In neither instance is the result remarkable, and it is extremely doubtful that humans would experience any effect at normal doses. The long term use of stevia would probably have a cardionic action, that is, would produce a mild, beneficial, strengthening of the heart and vascular system.

Antimicrobial Action: The ability of stevia to inhibit the growth and reproduction of bacteria and other infectious organisms is important in at least two respects. First, it may help explain why users of stevia enhanced products report a lower incidence of colds and flu, and second, it has fostered the invention of a number of mouthwash and toothpaste products. Research clearly shows that *Spreptococcus mutans, Pseudomonas aeruginos, Proteus vulgaris* and other microbes do not thrive in the presence of the non-nutritive stevia constituents. This fact, combined with the naturally sweet flavour of the stevia, makes it is suitable ingredient for mouthwashes.

Effects on Digestive System: In the literature of Brazil, stevia ranks high among the list of plants used for centuries by the "gauchos" of the southern plains to flavour the bitter medicinal preparations used in their "mate tea" (*Ilex paraguayensis*). Through much experimentation, these people learned that stevia made a significant contribution to improve digestion, and that is improved overall gastrointestinal function. Likewise, since its introduction in China, stevia tea, made from either hot or cold water, is used as a low calorie, sweet tasting tea, as an appetite stimulant, as a digestive aid, as an aid to losing weight and even for staying young.

Effects on Skin: One of the properties of a liquid extract of stevia, that has not been investigated experimentally, is its apparent ability to help clear up skin problems. The Guarani and other people who have become familiar with stevia report that it is effective when applied to acne, seborrhoea, dermatitis, eczema, etc. placed directly in cuts and wounds, more rapid healing, without scaring, is observed. (This treatment may sting for a few seconds, but this is followed by

a significant lowering of pain.) Smooth skin, softer to the touch is claimed to result from the frequent application of stevia poultices and extracts.

Effects on Reproduction: One effect on reproductive physiology, which must await further research, is a healing effect on the process underlying prostate disease.

OTHER NATURAL SWEETENERS

In addition to stevia, quite a few other natural products known for long as sweeteners with far better properties for our bodies than refined sugars or artificial sweeteners, are listed below.

- **Agave teuquilana:** Its nectar is sweet syrup, like honey, but a little thinner in consistency. It is a great honey replacement for diabetics and low-glycemic dieters due to its low glycemic index and thus blood sugar is not elevated. Agave nectar is made from its juice.
- **Barley malt syrup and powder:** A liquid sweetener that is reminiscent of molasses. Can be substituted for molasses in baking. Barley malt syrup metabolizes slowly in the body but does have calories. Diabetics and low-carb dieters should use it with caution.
- **Brown rice syrup:** It is liquid sweetener with the consistency of honey. It can be substituted for honey in baking. It has a unique caramel-like flavour that can be used to enhance a recipe, but it will disappear if used sparingly in a recipe. Brown rice syrup metabolizes slowly but does have calories and carbohydrates. Diabetics and low-carb dieters should use it with caution. Otherwise, it is fine for anyone who wants a whole-food natural sweetener.
- **Date sugar:** It is made by simply dehydrating dates and grinding them up into a rather coarse, granulated-type sugar. It contains fiber and nutrients, just as fresh dates do. Although, it will not dissolve very well in our cup of coffee it works very well, substituted cup-for-cup, in any baked-goods recipe that calls for brown sugar. Dates are 50 to 70 per cent sugar by weight. The supersweet Deglet Noor variety contains the same sucrose as sugar cane. The Halawy, Zahidi and Khadrawy varieties contain invert sugar composed of dextrose and levulose, similar to that in honey. Though fresh dates score low on the glycemic index, dried fruits always score higher, and dehydration would make it score higher still, as the sugar becomes more and more concentrated. So it’s not recommended for diabetes or those on a low-glycemic diet, but it’s great for anyone else who wants a very unrefined close-to-sweetener.

- **Evaporated cane juice:** "Evaporated organic cane juice" is that the juice of the same sugar cane used to make refined white sugar, but in it's natural state. Only the water is removed. As a whole food it still retains its vitamins and minerals. It also retains its natural balance of sucrose, glucose, and fructose instead of being straight sucrose. This is a dark brown sugar that contains molasses and has a slight caramel flavour.
- **Fruit spreads:** These are like jams or preserves, but sweetened with concentrated fruit juice syrup instead of sugar. Usually these syrups are made from grape, apple, pear, or pineapple juice, or some combination of these. These are all natural, unrefined fructose. They are delicious and taste even better than sugar sweetened variety because the sweetener is more harmonious in character with the natural fruit.
- **Honey:** Honey is "the nectar and floral exudations of plants gathered and stored in the comb of honeybees." It is a thick, sticky syrup that is 40% sweeter than sugar. It has a high glycemic index, so it is not recommended for diabetics or low glycemic dieters. Honey comes in a wide range of colours and flavours-darker honeys having stronger flavours and lighter honeys more mild flavours. Honey is in perfect edible form in its natural state, requiring no processing. It was one of our first sweetener and highly prized around the world for centuries. It was our primary sweetener until the industrialization of sugar cane in the mid-1800s. Honey has many health benefits, particularly when eaten raw.
- **Maple syrup and sugar:** It is a very sweet liquid sweetener, made by harvesting the sap from maple trees and boiling it down to a syrup. As continued boiling removes even more water, it turns into that nice, creamy maple sugar candy, and eventually into granules. It has its own unique flavour that is well-known and well-loved. Maple syrup and sugar has a very high glycemic index and will make our blood sugar jump. So if we are diabetic or on a low-glycemic diet. Stay away from maple syrup. It's fine for anyone else who wants a very low-processed natural sweetener.
- **Vegetable Glycerin:** it is derived from palm (coconut) oil. It is colourless, odourless, calorie-free, does not make blood sugar rise and is about half again sweeter than sugar. It is liquid used in many cosmetic preparations and in commercial food preparations, but is not yet widely sold as a sweetener.

- **Xylitol:** It is a naturally occurring sugar alcohol, not a sugar. The sweetener is found in many fruits, including berries, mushrooms and lettuce. It is not a strange or artificial substance to our bodies, but ia a normal part of everyday metabolism. Our body produce up to 15 grams of xylitol from regular food sources.

ADVANTAGES OF USING STEVIA

The advantages of using stevia over conventional sweetener are:

- Stevia leaves are 20-30x sweeter than sugar.
- Stevia leaves can be dried and stored.
- Stevia can be used in raw form.
- Stevia is short duration crop.Similarly stevia can be harvested 3-4 times a year for five years.
- Initial cost of establishment is high, 4,0000 plants per Acre and Rs 5 per plant.
- The yearly yield can be in the range of 4-5 tons stevia which can be sold @ 60-120 Rs per kilo of dried leaves.

CONCLUSION

India is not only one of the largest producer of sugar, but also one of the largest consumer of sugar. Sugarcane cultivation and processing requires a huge amount of land and human resource. Moreover, sugarcane cultivation involves higher amount of energy and inputs. In comparison, one ace of stevia cultivation would produce sweetener equivalent to 36 Acres of sugar cane. India being the largest consumer of cane sugar along with largest diabetic population in the world. Stevia is ideally poised to make significant contribution in satisfying the Indian demand of natural low calorie sweetener. As per WHO findings, this wonderful shrub has other medicinal virtues e.g. it regulates blood pressure, fights cavities, induces pancreas to produce more insulin, skin care, flavour inhancer and bactericidal agent, Ray, 2007.

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