

# Domestication of Non-Conventional Crops to Combat Human Health Diseases: A Review on Crop *Stevia Rebaudiana* in View of Pakistan as an Example

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**Abstract:** In developing countries health diseases such as diabetes and obesity are increasing at alarming rate. One of the major reason is an excessive use of sucrose (Sugar) obtained from the extract of *Sacharum Officinarum* and *Beta vulgare*. Sugar serves as a toxin if consumed in excessive amount and disrupt the body's usual hormonal cycles. Being integral part of our food system, it is not possible to eliminate the consumption of sugar from our routine life. Under such circumstances, there is a need to find an alternative sugar crop having less dreadful effects on the health of the human. *Stevia rebaudiana*, a zero or less caloric crop sweetener, is already widely being used as an alternative crop in many regions of the globe. The future of this plant is promising in Pakistan. However more research is needed to confirm its adaptability and economics in various regions of Pakistan. This review paper provides comprehensive information about taxonomy, history, origin, ecological requirement, botanical description, chemical properties, and importance of *Stevia rebaudiana*. The major aim of this review paper is to encourage the cultivation of *Stevia rebaudiana* as sugar alternative crop through awareness and acceptance of this crop among sugar producers and consumers as a new promising sugar crop.

**Keywords:** Crop domestication, ecological requirements, health diseases, food system, *Stevia rebaudiana*, sugar,

## I. INTRODUCTION

Genus *Stevia*, Asteraceae family, comprises about 230 different species involving both annual and perennial herbs, subshrubs and shrubs that mostly grow in mountainous areas, forests, river boarders and dry vallies (Frederico *et al.*, 1996; Guleria & Yadav, 2013). Of these 230 species only two species, *S. rebaudiana* and *S. phlebophylla*, possesses the relatively high amount of steviol glycosides (SGs) responsible for the sweet taste (Savita *et al.*, 2004b; Brandle & Telmer, 2007; Prakash *et al.*, 2008; Gupta *et al.*, 2013; Shivanna, 2013;).

*Stevia rebaudiana* is a perennial shrub mostly grown in tropical and sub-tropical climatic regions and is native to South Africa particularly Paraguay (Mizutani & Tanaka, 2002). From hundreds of years, its dry leaves have been used as natural sweating agent having relatively 7-15 % high concentration of steviol glycosides (Chaturvedula, *et al.*, 2011; Carakostas *et al.*, 2011). Amongst these steviol glycosides, Stevioside (60-70% of total SGs) and rebaudioside-A (15-20% of total SGs) are more important in spite of other SGs e.g. steviolbioside, rebaudioside-C, rebaudioside-D, rebaudioside-E, rebaudioside-F and ducloside-A (Bhimba *et al.*, 2010; Crammer and Ikan, 1986).

Nowadays, *S. rebaudiana* is getting more interest in several countries as an alternative sugar crop and is widely used in most of the part of the world by diabetic people or who need fewer calories. In 2009, highly purified steviol glycosides received the GRAS (Generally Recognized as Safe) status in USA (Jaworska *et al.*, 2012) and are also approved as food additive in USA, UK, China, Japan, Korea South America, Mexico, Indonesia and Canada (Bondarev *et al.*, 2003; FSANZ, 2008; Wolwer-Rieck, 2012; Dey *et al.*, 2013a).

Studies exposed the importance of *Stevia rebaudiana* as low or non-caloric, non-toxic and non-mutagenic ingredient (Sekihashi *et al.* 2002; Mizutani and Tanaka, 2002; Kim *et al.*, 2002) and its utilization throughout the world especially in USA, Spain and France as sugar Substitute as well as in beverages, wine making, fruit drinks, cosmetics and bakery products (Kinghorn, 2002; Gregersen *et al.*, 2004; Chatsudthipong & Muanprasat, 2009; Wolwer-Rieck *et al.*, 2010; Goyal *et al.* 2010; Stoyanova *et al.*, 2011). Production of *Stevia rebaudiana* is spread to

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different countries of the world including Canada and specific regions of Europe and Asia (Amzad-Hossain *et al.*, 2010; Gardana *et al.*, 2010).

Dry leaves of *Stevia rebaudiana* are 300 to 450 times more sweet than sucrose (Chang *et al.*, 2007; Yu and Shi, 2009; Silvia & Luciana, 2013) and also contain flavonoids, vitamins, tannins, phytosterols, minerals, alkaloids, hydroxycinnamic acid, essential oils and other miscellaneous compounds with antimicrobial as well as antioxidant properties (Tadhani & Subhash, 2006; Tadhani *et al.*, 2007; Darabpour *et al.*, 2010; Muanda *et al.*, 2011; Wolwer-Rieck, 2012; Lemus-Mondaca *et al.*, 2012; Belda-Galbis *et al.*, 2014;).

Consumption of dry leaves of *Stevia rebaudiana* positive influences the human health by regulating hypotension, enhancing antiglycaemic activity, preventing dental cavity, decreasing heart rate or improving digestion and gastrointestinal functioning (Lee *et al.*, 2001; Midmore & Rank, 2002; Jeppesen *et al.*, 2003; Gupta *et al.*, 2013). Sweeteners usually used such as table sugar and maple sugar are consist of glucose, sucrose and fructose primarily produced from sugarcane (*Sacharum Officinarum*) with a small contribution of sugar beet (*Beta vulgaris*) (Savita *et al.*, 2004a).

Main drawback of these sweeteners is that they highly contributes to calories and have dreadful effects on human health and body weight. Excessive energy from sucrose is stored by the body in the form of fat that ultimately increases the chances of several diseases like obesity as well as subsidizes to cancer, diabetes hypertension and cardiovascular diseases (Pearson, 200; Savita *et al.* 2004b; Bopp & Prince, 2001). During the year 2012-13, worldwide production of cane sugar was 180.837 MT and its consumption was 176.33 MT. The production of cane sugar has been of the order of 5.03 MT tones and that of beet sugar 0.033 thousand tones in Pakistan (PSMA, 2013).

Currently, about 0.382 billion people in the world are suffering from diabetes which might increase to 0.592 billion by year 2035 (Guariguata *et al.*, 2014) and in 2030 the seventh most leading cause of death will be diabetes (Kelly *et al.*, 2008). According to the International Diabetes Federation (IDF), about 6.713 million people suffers from the dreadful disease called diabetes in 2013 which will possibly increase to 12.798 million in 2035 (Guariguata *et al.*, 2014), whereas, Global Burden of Disease Study, rank Pakistan at number 9 out of 188 countries in terms of obesity (Ng *et al.*, 2014).

Most of western countries of the world consume products produced from medicinal or functional plants such as natural food increments or herbal tonics that promotes good human health is increasing and getting solid interest (Goldberg, 1994; Boue *et al.*, 2009). Such natural food products can be used as an appreciated tool in the avoidance and control of such diseases like obesity, cardiovascular disease, diabetes, cancer and other related diseases increasing drastically in many countries of the world. .

Researches revealed that secondary metabolites present in *Stevia rebaudiana* have no teratogenic, mutagenic or carcinogenic effects and doesn't have allergic reactions if consumed as a sweetener (Pol *et al.*, 2007). For this reason, use of *Stevia rebaudiana* as a low-calorie sweetener alternative to sucrose can led to the development of new natural sugar product thus improves many aspects of human health in the treatment of obesity, diabetes, hypertension and cardiovascular diseases by reducing calories (Chen *et al.* 2006; Boue *et al.*, 2009). Therefore, we presented a review paper on its potential as an alternative sugar crop in order to create awareness about this species among sugar producers and consumers for its acceptance thereby making contribution in enhancing its importance as a new promising sugar crop.

## **II. TAXONOMY**

*Stevia rebaudiana* belongs to the Class Magnoliopsida, Suborder Asteranae, Order Asterales, subtribe Ageratinae, and tribe Eupatorieae (Newell-McGloughlin, 2008; Guleria & Yadav, 2013; Kumari & Chandra, 2013). Spanish Botanist & Physicist, P. J. Esteve first discovered genus *Stevia* in the 16th century. Dr. Moisés Santiago Bertoni was the first person who discovers this plant in 1888 in Paraguay. *Rebaudiana*, the species name, credited in honor of the French Chemist Dr. Rebaudi, who first identified the sweetening substance in the plant, in 1905. Earlier, it was botanically classified as *Eupatorium rebaudianum* Bertoni but later it was changed to *S. rebaudiana* Bertoni. *Stevia rebaudiana* in Guarani language of its native area, Paraguay, is commonly known as “Caá hê-é” means sweet herb (Soejarto, 2002).

## **III. HISTORY**

Leaves of *Stevia rebaudiana* were firstly used as sweeten mate or as a general sweetening agent by native people of Paraguay and Brazil (Soejarto, 2002; Sehar *et al.*, 2008). M. S. Bertoni, a European Botanist, in 1887 first to

document *Stevia rebaudiana* (Schreiber, 2010) and French chemists Dr. Rebaudi in 1905, extracted different sweetening agents like rebaudioside & stevioside, the main sweet element in the form of white crystalline compound (Puri *et al.*, 2011). Subsequently, *Stevia rebaudiana* was utilized as a sweetening agent intended for food shortages during World War II when sugar again became unavailable (Small *et al.*, 2001; LeCroy, 2014).

In 1942, an unsuccessful attempt to establish *Stevia rebaudiana* as a crop was made in England. But in 1964, its first commercial cultivation was reported in Paraguay (Katayama *et al.* 1976; Lewis 1992). Afterward, great efforts were made by Sunida in 1968 to establish *Stevia rebaudiana* cultivation in Japan. In 1971, researches were conducted to evaluate the potential of *Stevia rebaudiana* in Japan (Crammer & Ikan, 1986) and today Japan is the major grower as well as marketer of *Stevia rebaudiana* in all over the world (Ramesh, 2006). Later on, it was introduced as a crop in a many countries including Brazil, Canada, Indonesia, Korea, Mexico, Tanzania and United States (Yadav *et al.*, 2011) Fors 1995). Presently, China is the largest producer of *Stevia rebaudiana* and its major market is in Japan with an estimated market value of around 2–3 billion yen/yr (Kinghorn & Soejarto 1985). *Stevia rebaudiana* is getting attention with the increase in demand for low carbohydrate and low calories alternative sugar crop in order to control obesity, diabetes and high blood pressure.

Nowadays, it is adapted as an alternative sugar crop in countries including Belgium, Central America, China, Japan, Mexico, Malaysia, Paraguay, Spain, South Korea and UK (Williams & Burdock, 2009; Puri *et al.*, 2011). It is expected that in future *Stevia rebaudiana* will overhaul the status of sucrose because of its safety and therapeutic values against several harmful ailments

#### **IV. ECOLOGY**

*Stevia rebaudiana*, native to the tropical regions of Brazil and Paraguay, occurs naturally in subtropical regions on the boundaries of marshlands or in grassland up to the elevation of 700 m (Yadav *et al.*, 2011). In the tropics regions it can be grown at altitudes up to 1500 m. *Stevia rebaudiana* can grow best in semi-humid sub-tropical areas with an annual rainfall of 1500-1800 mm, temperature of 21- 43°C for its normal growth (Huxley, 1992). It cannot tolerate extreme cold conditions and temperature below 9°C cease down the normal functions of *Stevia rebaudiana* (Singh & Rao, 2005).

*Stevia rebaudiana* can be grown in areas with wide range of soil having pH ranging from 6.5-7.5 (Chalapathi, 1999) but usually prefers a sandy soil, requiring a warm sunny position (Midmore & Rank, 2002). It needs a critical photoperiod of 12 hours to initiate flowering usually 40-60 days after flowering. However, increase in day-light duration promotes production of leaves which ultimately results in higher stevioside contents. *Stevia rebaudiana* shows better results when grown as a crop in fertile fields (Oddone B., 1999). It requires regular supply of water for its normal vegetative growth which might results in permanent wilting of *Stevia rebaudiana* leaves therefore it must soil must be well drained in order to prevent such losses (Chalapathi, 1999). An optimum ecological criterion for the cultivation of *Stevia rebaudiana* is shown in Table. 1.

An ecological study of *Stevia rebaudiana* shows that it can be successfully adapted as an alternative sugar crop in subtropical areas of Pakistan. These areas meet the ecological criteria for *Stevia rebaudiana* cultivation and untidy topography might help in the drainage of water which will diminish the risk of permanent wilting.

#### **V. BIOLOGY**

##### **5.1. Botany**

*Stevia rebaudiana* is a short day plant that can grow up to the height of 2-6 feet approximately forming a vigorous shallow root network (Woelwer-Rieck *et al.*, 2010). Its growth is totally dependent on existing weather conditions. Growth pattern of *Stevia rebaudiana* is divided into 4 different stages, germination and seedling establishment, vegetative growth, floral bud initiation, pollination to fertilization and seed growth and maturity (Ramesh *et al.*, 2006). *Stevia rebaudiana* form abundant stock of fibrous and filiform perennial roots that hardly reaches the land surface and have zero stevioside (Zaidan *et al.*, 1980; Ramesh *et al.*, 2006). Taiari *et al.*, 2004 stated that *Stevia rebaudiana* form a structure of fine roots near the soil surface and thick roots structure in deep zone. Stem of *Stevia rebaudiana* is annual, sub-ligneous and pubescent (Ramesh *et al.*, 2006). Its leaves are 3-8cm long and 1-2 cm wide having lanceolate to oblanceolate shape, with fine saw-like margin and are arranged alternatively (Dwivedi, 1999). Small sized self-incompatible florets (7–15 mm) of *Stevia rebaudiana* are white in color with purplish throat, arranged in irregular corymbs form having 2-6 florets (Dwivedi, 1999; Singh & Rao, 2005; Yadav *et al.*, 2011). Plant completes its flowering period within a month (Ramesh *et al.*, 2006). Seed of *Stevia rebaudiana* is an achene of 3

mm length has 20 feathery pappus (Pedroza Carneiro, 2007). Seed of *Stevia rebaudiana* are usually infertile thus it shows a very poor percentage of germination (Carneiro *et al.*, 1997; Lemus-Mondaca *et al.*, 2012).

### **5.2. Seed Germination**

Seeds of *Stevia rebaudiana* are smaller in size and it usually shows a very poor germination percentage (Singh & Rao, 2005). Thus, cultivation of *Stevia rebaudiana* through seeds is not an adequate approach because its germination varies greatly (Taware *et al.*, 2010; Yadav *et al.*, 2011). Lemus-Mondaca (2012) stated that one of the major limiting factors in the cultivation of stevia is poor seed germination. Therefore, the propagation through cutting and tissue culture is usually considered as an effective approach for cultivation of *Stevia rebaudiana* on a large scale (Chalapathi & Shivraj, 1997).

### **5.3. Propagation**

Traditionally, propagation of stevia was done through cutting, an instable, unreliable and less productive method, which takes a lot of time, labor and cost (Karim *et al.*, 2013). Hence, there is a crucial need to develop method for rapid multiplication of *Stevia*.

### **5.4. Cutting**

Propagules obtained as the result of stem cuttings usually shows unpredictable and unstable outcomes. Chalapathi (1999) reported that pre-treatment of cutting (15 cm) with Paclabutrastrol at the rate of 50-100ppm showed good effect on the sprouting and rooting of *Stevia rebaudiana*. In order to achieve good rooting results, cuttings from the main stem should be used for vegetative propagation and must be treated with growth regulators for adequate rooting (Bondarev, 1998; LeCroy, 2014). Therefore, in order to cop such type of restrictions associated with traditional propagation method of *Stevia rebaudiana* cultivation, development of micro-propagation through axillary buds and shoot tips played their effective role in its rapid multiplication.

### **5.5. In-Vitro Cultures**

For the preparation of adequate stock in short time, require enough labor and stem cuttings are required for the cultivation of *Stevia rebaudiana* on a large scale (Carneiro *et al.*, 1997). In this context, production of adequate plantlets is only possible through a reliable substitute technique such as in-vitro cultures e.g. shoot tip, axillary buds (Sivaram and Mukundan, 2003; Anbajhagan *et al.*, 2010; Taware *et al.*, 2010; Jagatheeswari and Ranganathan, 2012; Sabah and Rasha, 2013). Micro-propagation helps in recovering a genetically stable and true-to-type progeny and also helps in restoring important Germplasm (Thiyagarajan & Venkatachalam, 2012). Reports on the in-vitro cultures exist as a convenient method through leaves, nodes, internodes and shoot tips for large scale cultivation (Salim *et al.*, 2006; Thiyagarajan & Venkatachalam, 2012).

## **VI. STATUS IN PAKISTAN**

*Stevia rebaudiana*, a natural sweet herb, is cultivated as an alternative sugar crop and cash crop in different countries on the globe. Cultivation of *Stevia rebaudiana* on a large scale faces a problem because of its poor seed germination. Therefore, unavailability of mechanized production technology is biggest hurdle in the domestication of *Stevia rebaudiana* (Savita *et al.*, 2004a). In Pakistan, *Stevia rebaudiana* was introduced through tissue culture technique during the year 2003 (Talha *et al.*, 2012).It cultivation is still limited to research and is not cultivated as a cash crop or alternative sugar crop (Fazal *et al.*, 2011).Rafiq *et al.* (2007) instead of having a very diverse weather conditions still there is no literature on the cultivation as well as in-vitro propagation of *Stevia rebaudiana* on commercial level of *Stevia rebaudiana* in Pakistan. However, Pakistan Agricultural Research Council (PARC) and Punjab Agricultural Research Board (PARB) have approved several research proposals for the present and future scenarios on the development and dissemination of information about *S. Rebaudiana* cultivation in various regions of Pakistan

## **VII. PRODUCTION TECHNOLOGY**

### **7.1. Sowing Time**

Nursery of stevia can be prepared usually through seed, cuttings and tissue culture in Pakistan.Plants prepared from seeds are transplanted to field after 2 months, whereas, seedlings from tissue culture or cuttings can be transferred after one month. For spring cultivation, nursery should be sown in December-January and should be transplanted to field in February. For Rabi cultivation, nursery should be sown in August-September and transplanted to field in October-November.

## **7.2. Nursery Sowing**

For good germination, only dark brown or black colored seeds should be used for nursery sowing. Seeds should be sown in plastic bags or pots filled with equal amount of thoroughly mixed sand, silt, clay and organic matter. After sowing, cover the seeds with a thin layer of soil, and then sprinkle some water to facilitate germination. Bags or pots should be covered in order to conserve moisture. Light Irrigation with the help of sprinkler should be done at the suitable interval of one to two weeks until maximum germination is obtained. For cuttings, stem of 4 cm height containing axillary buds should be sown in a plastic cup up to a depth of 1 to 2 cm and then irrigate it. After that, cover the plastic cups with polythene sheet and place it at 15-20°C. When the cuttings get ready, transfer it to the field.

## **7.3. Land Preparation**

Field should be plowed or cultivated twice to prepare a fairly, firm and smooth planting surface. The soil should be well pulverized and thoroughly mixed with organic matter, potassium and phosphorus fertilizers.

## **7.4. Nursery Transplantation:**

After 15 days, remove the plastic bags containing cuttings from the pots and place them under a covered place to make them hardy. This is necessary for the young seedlings to tolerate the climatic conditions and grow further. To obtain optimum plant population the seedlings should be planted at row to row distance of 45 cm and plant to plant distance of 30 cm. After the completion of transplantation, irrigate the field to ensure optimal crop stand.

## **7.5. Fertilizer & Irrigation Scheduling:**

Two bags of Urea (50 Kg per bag), one bag of DAP (50 Kg per bag), and one bag of Ammonium Sulphate (50 Kg per bag) should be applied to obtain good economic leaves yield. Field should be light irrigated 1-2 times in a week. In summer season, especially June and July, light irrigation should be applied. Excessive water around the root for a long period may result in the yellowing of leaves due to suffocation and increase the chances of fungus diseases attack.

## **7.6. Disease & Insect**

Stevia is usually attacked by termites, sucking insects (Thrips, Aphids, and Jassids), American worm, and mealy bug. But these insect doesn't cause severe damage to Stevia. In case of severe attack, following chemicals should be used

### **7.7. Termites**

Termites usually attack in dry conditions. For the control of termites Chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) 40.I.C at the rate of 2 liters per acre should be applied with irrigation water.

### **7.8. Sucking Insects**

For the control of sucking insects, Imidacloprid (200 SL) at the rate of 200ml or Acetamiprid (20SP) at the rate of 150 gm in suitable quantity of water should be sprayed. For controlling mealy bug, Profenofos at the rate of 1 liter per acre should be sprayed.

### **7.9. American Worm**

American worm should be controlled by spraying 150 ml per acre Laporan in 100 liters of water.

### **7.10. Root Rot**

400 g Topsin in 100 liter of water should be sprayed near the roots. Because it usually attack on the roots of emerging seedlings

### **7.11. Harvesting**

Leaves should be harvested before flowering in order to obtain high concentration of sweetening agents known as "Steviol glycosides".

### **7.12. Yield**

18-22 mounds dry leaves per acre.

### **7.13. Reference**

Akram *et al.* (2013)

### VIII. CHEMICAL & NUTRITIONAL PROPERTIES

Apart from these sweetening glycosides, *Stevia rebaudiana* is famous because of its chemical and nutritional properties i.e. amino acids, minerals and phytochemicals (Chu *et al.*, 2000; Edeoga *et al.*, 2005; Singh & Rao, 2005). Leaves of *Stevia rebaudiana* also contain flavonoids, gums, inorganic matter, pigments, sterbins A to H, triterpenes and volatile oil constituents (Brandle *et al.*, 1998). Dry leaves of *Stevia rebaudiana* provide a strong basis of carbohydrate, protein, and crude fiber in maintaining good human health (Savita *et al.*, 2004a; Abou-Arab *et al.*, 2010; Mishra *et al.*, 2010; Goyal *et al.*, 2010; Lemus-Mondaca *et al.*, 2012).

Abou-Arab *et al.* (2010) stated that *Stevia rebaudiana* act as a good source of nutrients/minerals (Table. 2.) e.g. Phosphorus, Potassium, Sodium, Calcium, Iron, Zinc, and Magnesium as well as a valuable source of essential amino acids (Table. 3.), required for improving defense mechanism of body, regulating and maintaining several metabolic processes (Savita *et al.*, 2004a; Lemus-Mondaca, 2012). Leaves of *Stevia rebaudiana* comprises number of different types of essential oils (Table. 4.), usually referred as “Life force” of a plant (Yadav & Guleria, 2012).

### IX. THERAPEUTIC ACTIVITIES

Addition to lessor Non-caloric sweetness, *Stevia rebaudiana* also have other some therapeutic properties accompanied by related compounds such as sweet diterpene glycosides (Rebaudioside A, Steviol and Isosteviol), alkaloids, flavonoids, amino acids, hydroxycinnamic acids (Caffeic, Chlorogenic), lipids, water-soluble oligosaccharides, free sugars, essential oils and trace elements (Komissarenko *et al.*, 1994; Hossain, 2012). These compounds possess anti-diarrheal, anti-hypertensive, anti-cariogenic, anti-carcinogenic, anti-inflammatory, anti-diabetic, diuretic, and immune-modulatory properties (Goyal *et al.*, 2010). As compare to the other natural and artificial sweeteners, *Stevia rebaudiana* possesses significant pharmaceutical values (Anton *et al.*, 2010).

#### 9.1. Anti-Diabetic Activity

Diabetes is usually caused by insulin abnormality, pancreatic dysfunction and glucose excess (Leahy, 200; ADA, 2010). Diabetic nephropathy is usually done in the treatment of chronic diabetes but it leads to development and spread of small blood vessels which ultimately leads to cardiovascular diseases (Ziegler, 2008). Beside these effects, none of the single oral anti-diabetic agent can maintain euglycemia and other cardiovascular diseases on long term basis (Shivanna *et al.*, 2013). Under such conditions, Stevioside can be used as an alternative medicine and also as an excellent source for nutraceuticals (Dey *et al.*, 2013b). Stevioside has a significant effect on glucose metabolism by reducing blood glucose level in patients suffering from type 2 diabetes Moreover, by the action on beta cells it enhances the secretion of insulin thus helps in the efficient recovery of diabetic patients (Dyrskog *et al.*, 2005). The main activity of this anti-diabetic agent is to energize the beta cells of pancreas, enhance insulin sensitivity and stimulate supplementary insulin synthesis (Jeppesen *et al.*, 2000). Leaf Extracts of *Stevia rebaudiana* also increase ability of glucose tolerance in humans (Chen *et al.*, 2005; Gregerson *et al.*, 2004; Anton *et al.*, 2010). In South America, extract of *Stevia rebaudiana* is being used from many decades as an anti-diabetic agent (Jeppesen *et al.*, 2002).

#### 9.2. Anti-Oxidant Activity

Energy production for human biological metabolism is totally dependent on the oxidation process. During this process, molecular oxygen, by generating reactive oxygen species (ROS) reduces into water such as O<sub>2</sub><sup>-</sup>, OH<sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, RO<sub>2</sub><sup>·</sup>, ROO<sub>2</sub><sup>·</sup> and R. (Apel & Hirt, 2004). These ROS are involved in the initiation of many diseases like arthritis, cancer, cardiovascular disorder, diabetes, inflammation, immune deficiency, neurologic disorder etc (Parejo *et al.*, 2002; Hou *et al.*, 2003; Valko *et al.*, 2007; Kim *et al.*, 2011). For the assassination of such conditions, natural foods and medicinal plants, having the ability to scavenge free radicals, are utilized as an anti-oxidant (Hou *et al.*, 2003; Galvez *et al.*, 2005). Stevioside present in leaves of *Stevia rebaudiana* may act as a potential source as a natural antioxidant (Jahan *et al.*, 2010). Diversity of antioxidants including, opigenin, kaempferol and quereitrin are present in leaf extract of *Stevia rebaudiana* that have the inhibiting ability against damage of DNA strand. Isosteviol, derived from stevioside, can reduce the generation of ROS by inhibiting the angiotension-II-induced cell proliferation and endothelin-1 secretion (Ghanta *et al.*, 2007; Stoyanova *et al.*, 2011). Isosteviol also improves the antioxidant defense in the adipose tissue and the vascular wall by inhibiting the atherosclerotic plaques development or by stabilizing of induction plaque (Geeraert *et al.*, 2010). A phytochemical namely polyphenols play an important role in enhancing the antioxidant activity of this plant (Chatsudthipong & Muanprasat, 2009; Ahmad *et al.*, 2010)

#### 9.3. Anti-Microbial Activity

Plant extracts and phytochemicals e.g. terpenes, tannins and flavonoids, present in *Stevia rebaudiana* can play a significant role as an anti-microbial agent in the cure of several clinical problems (Dabur *et al.*, 2004; Debnath,

2008; Darabpour et al., 2010). These phytochemicals are present in rich amount and are aesthetic, anti-inflammatory, austroinullin and cardio-tonic in nature (Hossain, 2012). Octa-acetylombuoside, ombuine and retusine derived from stevioside show antimicrobial agent against few types of gram-positive & gram-negative bacteria (Tomita et al., 1997). Vitery et al. (2010) verified this statement by detecting that leaves extract of *Stevia rebaudiana* showed inhibitory effect on gram positive bacteria (*B. subtilis*, *S. aureus*, *M. luteus*, *B. megaterium*), Gram negative bacteria (*S. marcescens*, *P. aeruginosa*, *E. coli*, *P. vulgaris*) and fungus (*R. oligosporus* and *A. niger*). However, it also has inhibitory effect on the binding abilities of several antibodies (Takahashi et al. 2001). Siddique et al., (2014) found that leaves extract of *Stevia rebaudiana* containing n-hexane have dreadful effect on several animal pathogens (*S. aureus*) and is influential enough in controlling several food spoiling fungus (*A. niger*) & Bacteria (*S. aureus*). Thus, it can be used as natural food preservative ingredient.

#### **9.4. Anti-Cariogenic Activity**

*Stevia rebaudiana* as a sweetener possesses an anti-cariogenic properties (Abu-Elteen, 2005; Chatsudthipong and Muanprasat., 2009). *Streptococcus sobrinus* is usually considered as a major agent involved in the development of all tooth decay and gingivitis (Okada et al., 2005). However, portion of *S. mutans* and *L. acidophilus* present in the plaque and saliva are directly responsible in increasing the incidence and activity of tooth decay (Thylstrup & Fejersko, 2000). Leaf extract of *Stevia rebaudiana* obstructs the cariogenic property of *Streptococcus sobrinus* (Brambilla et al., 2014) and found effective along with hexane impeding the growth of *S. mutans* and *L. acidophilus* and retard plaque deposits (Contreras, 2013).

#### **9.5. Anti-Hypertensive Activity**

*Stevia rebaudiana* is also considered as an effective, reliable and alternative therapy in lowering the blood pressure level of patients suffering with hypertension (Brahmachari et al., 2011). Stevioside extracted from the leaves of *Stevia rebaudiana* perform an activity of complete vasodilator and has positive role in hypertension for its vasodilator activity (Goyal et al., 2010). It acts as anti-hypotensor which causes vasorelaxation by inhibiting the Ca<sup>2+</sup>-influx in the blood vessels (Liu et al., 2002; Ulbricht et al., 2010). It induces diuresis and natriuresis without disturbing the renal plasma flow and cardiac frequency (Melis et al., 2009; Brahmachari et al., 2011). But comparable to other anti-hypotensive drugs its hypotensive effect is not even better than them (Liu et al., 2002). Stevioside at the rate of 0.75 – 1.5 g/ day reduces the systolic blood pressure by 10 – 11 mmHg and diastolic blood pressure by 6-14 mmHg within the 1st week of treatment (Ferri et al., 2006; Maki et al., 2008).

#### **9.6. Anti-Carcinogenic Activity**

Paul et al. (2012) stated that leaf extract of *Stevia rebaudiana* action as an inhibitor in preventing the initiation and promotion of tumors. Stevioside, rebaudioside A & C and dulcoside A possesses analogous activities in the promotion of TPA (12-O-Tetradecanoylphorbol-13-Acetate), effective cancer therapeutic agent, like other triterpenoids used in the treatment of various types of Cancer (Yasukawa et al., 2002; Raskovic et al., 2004).

#### **9.7. Anti-Diarrheal Activity**

Stevioside presents in the dry leaf extract of *Stevia rebaudiana* obstruct the contraction and relaxation of smooth intestinal muscle linked with hypermobility-associated diarrhea (Shiozaki et al., 2006). Additionally, the dihydroisosteviol, assumed as a CFTR inhibitor which might be used in the formation of anti-diarrheal agent, lessens the discharge of intestinal fluid (Brahmachari et al., 2011).

#### **9.8. Anti-Inflammatory & Immunomodulatory Activity**

Inflammation, either acute or chronic, is a usual response in mammalian tissue in which outer covering of a skin change in the form of painful swell due to injury or any other agent (Jain et al., 2011). ROS act as an inflammatory mediator in extending inflammatory responses and cell injury (Garcia et al., 2003). The phytochemicals present in the extract of *Stevia rebaudiana* possesses antioxidant properties which help in reducing inflammation (Bairagi et al., 2012). Stevioside reduces the production of inflammatory mediators by interfering with signaling pathway (Boonkaewwan et al., 2006). Arya et al., 2012 stated that the methanolic extract of *Stevia rebaudiana* showed potential as an anti-inflammatory agent.

### **X. WOUND HEALING ACTIVITY**

Dry leaf extract of *Stevia rebaudiana* is used in eliminating blemishes, cleansing acne, elasticizing skin and fastening of wound healing process if few drops are applied on cuts and scrapes as topical wound dresser (Barathi, 2003; Bolaños & Ramírez, 2007).

## XI. NEUROLOGICAL ACTIVITY

Leaf Extract of *Stevia rebaudiana* possesses the ability to create liveliness and comfort in addition to decrease in the desires of eating sweets, fast food, consumption of tobacco and alcoholic drinks (Durán et al., 2012. Sharma et al. (2010) observed that stevioside present in the *Stevia rebaudiana* possesses anti-amnesia properties asserting a protective effect on memory deficits.

## XII. OBESITY CONTROL

Most of the bakery, beverages and confectionary products are overloaded with sucrose which ultimately leads to overweight or obesity (Murphy, 2001). *Stevia rebaudiana* enclose no or less calories and is considered ideal for people aiming to loss or maintain their weight in the form dietary supplement (Park & Cha, 2010). Steviol glycoside extracted from leaves of *Stevia rebaudiana* fulfills the desire of sweet taste without gaining extra weight. Use of *Stevia rebaudiana* in bakery, beverages and confectionaries will help in the loss and maintenance of weight much easier than before (Bharat et al., 2013).

## XIII. UNIVERSAL TONIC

Stevioside has a shielding influence on the degradation of vitamin C in comparison to other sweeteners (Stoyanova et al., 2011). *Stevia rebaudiana* is also approved as a nutritional increment or an herb but not as a sweetener by USFDA (Stancik & Scholar, 2005). Proteins, phytochemicals, and minerals present in *Stevia rebaudiana* are crucial in the protection of good health. Leaf extract of *Stevia rebaudiana* is helpful as alcohol abuses, diuretic, digestive aid and as a food additive (Bharat et al., 2013).

## XIV. STEVIOL GLYCOSIDES VS. SUCROSE

In comparison with other sweeteners, nutrient composition of *Stevia rebaudiana* leaves maintain its status as a low caloric sweetener of energy value in *Stevia rebaudiana* is 27 kcal per 100gram which is less than others (Anonymous, 2000). Medical evidences show that excessive use of sugar (sucrose) obtained from sugarcane affects human health by the addition of calories (WHO; 2003). Sucrose, usually considered as high caloric, has a tendency to intensify fat deposition. Diseases associated with excessive use of sugar leads to several metabolic disorders like diabetes due to increase in liver glucose production and raises in blood level, blood cancer, cognitive decline and initiation of aging process due to damage of lipids, proteins and DNA (Lustig, 2010; Tappy, 2010).

Excessive use of sucrose exerts similar damage to liver like alcohol because alcohol is obtained from sugar fermentation (Lustig, 2010). In this context, *Stevia rebaudiana* is a totally natural herb with no calories and no adverse effects on the body. Savita et al. (2004a) stated that 1gm *Stevia rebaudiana* in 100ml of water is equal to 20gm of sucrose in 100ml water in term of sweetness. Apart from this, Steviol glycosides extracted from the leaves of *Stevia rebaudiana* are anti-carcinogenic, anti-cariogenic, anti-diabetic, anti-diarrheal, anti-inflammatory, anti-hypertensive, anti-microbial, anti-oxidant, diuretic, digestive aider, immune-regulator, vasodilator and alcohol abuse in nature (Jaitak et al., 2008; Lemus-Mondaca et al., 2012). *Stevia rebaudiana* also restrict the carving of sweets and help limiting calorie consumption in the diet (Savita et al., 2004a).

## XV. STEVIOL GLYCOSIDES VS. OTHER NON-CALORIC SWEETNERS

Mostly there are two types of non-caloric sweeteners i.e., natural and artificial sweeteners. Disadvantages of artificial sweeteners are much more than their advantages. Artificial sweeteners including aspartame, saccharin, glycyrrhizin and cyclamate are associated with phenylketonuria, blood cancer, bladder cancer, pseudo-aldosteronism and testicular atrophy along with dreadful cardiovascular effects respectively (Butchko et al., 2001; Pearson 2001; Bopp and Price, 2001, Yadav & Guleria, 2012). Some other commercially used less or non-caloric sweetening compounds are e.g. glycyrrhizin, mogroside, phylloolulcin, thaumatin, xylitol, and stevioside (Kim & Kinghorn, 2002; DuBois & Prakash, 2012). Among all these artificial or natural sweeteners, Steviol glycosides, a group of diterpene glycoside, are the best choice as an alternative non-caloric sweeteners having about 10 different natural sweetening agents, which are about 150-300 times sweeter than sucrose (Table 5) with no effect on human health (Dacome et al., 2005). Although, Steviol glycosides are present in all plant organs but their concentration is high only in leaves of *Stevia rebaudiana*. Amount of Steviol glycosides present in the leaves of stevia is shown in Table. 6.

## XVI. ROLE IN FOOD INDUSTRY

Use of sugar formulated dietary in routine life increases the rate of several complication like cancer, diabetes, enzymatic dysfunction, obesity, interferes in Ca<sup>2+</sup> and Mg<sup>2+</sup> absorption and cardiovascular diseases (Vartanian,



2007; Gibson, 2008; Vasanti, 2010). Increased rate of such complications grabbed the attention of scientist & researchers toward the development and domestication of new alternative food crops. Therefore, use of alternative less or non-caloric natural sweeteners can be a suitable replacement of sugar as a food ingredient. In this context, Stevia rebaudiana can be used as a substitute source of less or non-caloric natural sweetener. Leaves of Stevia rebaudiana is considered as a reliable basis of protein, carbohydrates and crude fibre (Braz de Oliveira et al., 2011). Nowadays, Stevia rebaudiana is becoming a major source of high potency natural sweetener used as substitute for saccharose in the production of low or less caloric natural food products and in future it can also be used as anti-diabetic, anti-carcinogenic, anti-hypotension pharmaceutical product (Savita et al., 2004a; Midmore & Rank, 2006; Pol, 2009; Goyal, 2010).

Steviol glycosides present in the leaves of Stevia rebaudiana are becoming famous with time and are available in market as an alternative food supplement in many industrial products e.g. confectionaries, coffee, fruit juices, ice creams, jams, sweets, soft drinks, porridge, tea and is also used as color enhancers (Goyal et al., 2010). Steviol glycosides, stevioside & rebaudioside A are the primary ingredients used in food and the product containing higher concentration of rebaudioside A is usually referred as Rebiana (Carakostas et al., 2008). In most of the parts of world, steviol glycosides are replacing sucrose as a substitute food ingredient in numerous food industries like cereals, yoghurt, pickles, candy, soybeans and soya sauce, and seafood's (Wallin, 2007; Koyama et al., 2003; Amzad-Hossain et al., 2010; Goyal et al., 2010). In 2008, the WHO/FAO Expert Committee on Food Additives (JECFA) and US Food and Drug Administration (USFDA) declared the use of steviol glycosides in defined range of food and beverages industries (Contreras, 2013). In 2009, France approved the use of rebaudioside A as a natural sweetener (Yadav & Guleria, 2012). In this context, European Commission in 2011 allowed the use of steviol glycosides as food supplement in order to establish maximum content for foods and beverages (Stoyanova et al., 2011).

One of the major problems associated with steviol glycosides is bitterness aftertaste. Michalik et al., 2010 found that alkaloid iminosugar, steviamine, is responsible for the bitter taste of steviol glycosides. Many food companies are wishing to use Steviol glycoside as a sweetener, but its bitter taste is creating hurdle for its wide spread use. Therefore, food companies are reluctant to explore the benefits of natural sweetness of Stevia rebaudiana. If the problem of bitter taste is overcomes then not a single natural or artificial sweetener will able to grab the sweetening status of Stevia rebaudiana. This may be achieved by cultivating this crop on different kinds of soils, by using different combinations of fertilizers and by using different molecular techniques which could affect its chemical properties to lessen its bitterness.

The suitability of a food can be described in different aspects by which it is cooked and handled (Savita et al., 2004a). Leaf powder of Stevia rebaudiana can be used as a sweetener in different food items. The bulk density of Stevia rebaudiana is lower as compare to other cereals and pulses which makes it an important factor for child feed products e.g. porridge, biscuits, cookies etc.

## **XVII. POTENTIAL IN AGRICULTURE**

Leaves of Stevia rebaudiana possesses certain insecticidal property by showing resistant against aphids. For the researchers & farmers, Stevia rebaudiana represents a new opportunity for research but still a lot of information about the optimum production technology is required for its successful establishment as a crop in Pakistan. Furthermore, it can be utilized as a source for the production of new metabolites in future (Hawke, 2003).

## **XVIII. SAFETY PROFILE**

Two major steviol glycoside, present in the leaves of Stevia rebaudiana are Rebaudioside A & Stevioside, used as alternative natural sweeteners (Wheeler et al., 2008). Earlier, Filho et al. (1989) and Melis (1999) stated that both these steviol glycosides have negative effect on the reproductive ability in male by reducing testosterone levels and sperm count. But, recent studies on the reproductive and general toxicity of Rebaudioside A revealed that Stevia rebaudiana is safe even if intake at high level as a natural sweetener (Brunswick 2008; Williams & Burdock, 2009; Qing, 2010). Investigations on these steviol glycosides showed that they also lack mutagenic, genotoxic prospective and are non-allergic and non-toxic (Geuns et al., 2004; Rajab et al., 2009). Patients suffering from diabetes intending to maintain their sugar level and from obesity aiming to lose their weight by escaping sucrose from their routine life can safely use stevioside as an alternative sugar without making their life tasteless.

Studies revealed that if sucrose is replaced with such type of steviol glycosides like Rebaudioside A & Stevioside in our routine life then it will help in eliminating the risk of diabetes, obesity, cardiovascular and other harmful diseases from Pakistan.

### XIX. CONCLUSION

*Stevia rebaudiana* is a newly emerging alternative sugar crop in many regions of the globe. As compare to other natural and artificial sugars *Stevia rebaudiana* is highly nutritious, delicious, and low or non-caloric promising sugar crop. Leaves of *Stevia rebaudiana* contain higher concentration of steviol glycosides and are 150-300 times sweeter than sucrose with no dreadful effect on the human health. Apart from this, these steviol glycosides are anti-diabetic, anti-hypertensive, anti-oxidant, anti-microbial, anti-inflammatory, anti-carcinogenic, anti-cariogenic, anti-diarrheal, immune-regulator, vasodilator, diuretic, digestive aider, and alcohol abuse in nature. Moreover, these Steviol glycosides can be used as a skin tonic, tobacco carving, food supplement and food preservative. Keeping in view the importance of *Stevia rebaudiana* awareness should be created among sugar producers and consumers about the nutritional and pharmacological properties of *Stevia rebaudiana* as a natural alternative sweetener. Furthermore, to use *Stevia rebaudiana* as an alternative sugar crop which will aid in the development of new research project in term of production technology, steviol glycoside extraction and improvement in quality of steviol glycoside in Pakistan. The increasing mortality rate due to cancer, diabetes, obesity and cardiovascular diseases can be controlled to some extent by the use of these steviol glycosides. Therefore, we conclude *Stevia rebaudiana* as an ideal candidate as an alternative sugar crop in Pakistan and further researches should be planned on its cultivation and pharmaceutical uses.

**Table1.** Classification and Optimum Ecological Requirement for the Cultivation of *Stevia rebaudiana* (Adriana *et al.*, 1996; Jayaraman *et al.*, 2008; Madan *et al.*, 2010)

Classification		Requirements for Cultivation	
Technical name	<i>Stevia rebaudiana</i>	Latitude	Between 22° 24°
Genus	<i>Stevia</i>	Temperature	15°C - 35°C
Family	Asteraceae/Compositae	Soil pH	5.5-6.0 (Slightlyacidic)
Sub-family	Asteraceae	Soil Type	Sandy loam Loam
Common Name	<i>Stevia</i> , Sweet leaf, Sugar Leaf, Candy Leaf, Sweet Honey Leaf, Rebiana.	Rainfall	1200-1700mm
Synonyms	<i>Eupatorium rebaudianum</i> Bertoni	Climatic Zone	Semi TropicalTemperate
No. of Chromosomes	2n=22		

**Table2.** Nutritional composition of *Stevia rebaudiana* reported by various authors

Nutrient composition	Savita <i>et al.</i> , 2004a	Tadhani and Subhash, 2006	Goyal <i>et al.</i> , 2010	Kaushik <i>et al.</i> , 2010	Serio , 2010	Abou-Arab <i>et al.</i> , 2010	Atteh <i>et al.</i> , 2011	Yadav and Guleria, 2012	Gasmalla <i>et al.</i> , 2014
Moisture (g/100g)	7	-	4.65	7.7	-	5.37	-	-	10.73
Energy (kcal/100g)	270	-	-	-	-	-	-	-	-
Protein (g/100g)	10	20.4	11.2	12	11.2	11.4	16	12-20.4	13.68
Fat (g/100g)	3	4.34	1.9	2.7	5.6	3.73	2.6	2.7-4.34	6.13
Carbohydrate (g/100g)	52	35.2	-	-	53	61.9	-	35.2	63.1
Ash (g/100g)	11	13.1	6.3	8.4		7.41	15.5	13.12	12.06
Crude fiber (g/100g)	18	-	15.2	-	15	15.5	6.8	-	5.03
Calcium (mg/100g)	464.4	1550	544	722	600	17.7	8.2	-	-
Phosphorus (mg/100g)	11.4	350	318		318	-	2.6	-	-
Potassium (mg/100g)	1800	2510	1780	839	1800	21.15	17.3	-	-
Iron (mg/100g)	55.3	36.3	3.9	31.1	3.9	5.89	366	-	-

Sodium (mg/10g)	190.0	160	89.2	32.7	-	14.93	0.7	-	-
Magnesium (mg/100g)	-	-	349	-	500	3.26	2.4	-	-
Zinc (mg/100g)	-	6.39	1.5	-	-	1.26	20	-	-

**Table3.** Concentration of Amino Acids in Leaf Extract of *Stevia rebaudiana*(Li et al., 2011)

Kind of Amino acids	Name of amino acids	Percentage (%)
Essential amino acids	Arginine	0.796
	Histidine	0.337
	Isoleucine	0.716
	Leucine	1.288
	Lysine	1.050
	Phenylalanine	0.867
	Proline	1.671
	Threonine	0.744
	Valine	0.935
Non essential amino acids	Alanine	0.941
	Asparagine	1.658
	Glutamine	1.894
	Glycine	0.846
	Serine	1.036
	Tyrosine	0.485

**Table4.** Chemical composition of the essential oils (Siddique et al., 2012) and Functional properties of leaf powder of *Stevia rebaudiana*(Savita et al., 2004a)

Chemical Composition of Essential Oils		Functional properties	
Essential Oil	(%)	Properties	Value
Cyclopentasiloxane	0.88	Bulk density	0.443 g/ml
Geranyl vinyl ether	0.14	Water absorption capacity	4.7 ml/g
Cyclohexasiloxane	4.4	Fat absorption capacity	4.5 ml/g
Indole	0.17	Emulsification value	5 ml/g
Caryophyllene	0.08	Swelling	5.01 ml/g
(-)-Spathulenol	2.21	Solubility	0.365 ml/g
Caryophyllene oxide	1.23	pH	5.95
Santalol, cis alpha pyranomenthol	0.07		
$\beta$ -Guaiene	0.32		
Ledene oxide-(II)	0.21		
$\alpha$ -Cadinol	2.98		
Aristolene epoxide	0.18		
Silanediol	4.49		
Estra-1,3,5(10)-trien-17-ones	1.92		

**Table5.** Sweetening strength of Steviol glycosides as compare to sucrose (Puri et al., 2011)

Stevioside Glycosides	Kinghorn and Soejarto, 1985	Crammer and Ikan, 1987	Kolb et al., 2001	Geuns , 2003	Pol et al., 2007	Gardana et al., 2010	Goyal et al., 2010	Atte h et al., 2011	Jaworska et al., 2012	Yadav and Guleria, 2012
Stevioside (g/100g)	5-10	3-10	3.78-9.75	4-13	43.1-79.6	5.8	9.1	6.5	2.0	4-14
Steviol (g/100g)	-	-	-	-	-	-	-	-	0.70	-
Steviol bioside (g/100g)	-	-	-	Trace	0.3-3	-	-	-	1.2	> 0.4
Rebaudioside-A (g/100g)	2-4	1.0	1.62-7.27	2-4	7.6-9.9	1.8	3.8	2.3	5.0	2-4

Rebaudioside-B (g/100g)	-	-	-	Trace	0.02	-	-	-	0.50	-
Rebaudioside-C (g/100g)	1-2	-	-	1-2	0.5-6	1.3	0.6	-	2.0	1-2
Rebaudioside-D (g/100g)	-	-	-	Trace	0.4	-	-	-	3.3	> 0.4
Dulcoside-A (g/100g)	0.4-0.7	0.2	-	0.4-0.7	0.2-0.4	-	0.3	-	1.0	0.4-0.7

**Table6.** Amount of sweet glycosides in *Stevia rebaudiana* leaves reported by various authors

Steviol glycosides	Sweetening Strength
Stevioside	150-300
Rebaudioside-A	350-450
Rebaudioside-B	300-350
Rebaudioside-C	50-120
Rebaudioside-D	200-300
Rebaudioside-E	250-300
Rebaudioside-F	ND
Steviol bioside	100-125
Dulcoside-A	50-120

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