Stevia *rebaudiana*; a precious gift of nature, it's therapeutic values and uses

Momna Abbasi, Muniba Zahid, Iqra Mukhtar, Kainat Raja, Iqra Javed, Hafiza Sana Zaid, Tayyaba Tariq, Parwasha Mubarik, Muqaddas Naeem, Kinza Khan, Hansa Zeb, Shazia Erum[2] and Arifa Khan*.

1.Department of Zoology, Government Viqar-un-Nisa Post Graduate College, Rawalpindi.

2.Bioresource Conservation Institute (BCI), PGRI Building,National Agricultural Research Centre (NARC)

***Corresponding author's email:** arifa.phdbt55@iiu.edu.pk

Abstract:

Stevia rebaudiana, a well-known medicinal plant, is a precious gift of nature. It is known for its non-caloric, bio-sweetening, and medicinal value. A herbaceous and perennial plant of the genus Stevia, Cav. It consists of approximately 250+ species of herbaceous, shrub, and sub-shrub plants. High content of protein, some essential minerals, and carbohydrates all indispensable amino acid are present in leaves of stevia, except tryptophan, folic acid, vitamin C and some bioactive chemical constituents as well as linolenic and palmitic acids. Two varieties of Stevia rebaudiana Bertonic prepared for cultivation in Mexico, their extract were screened and contained some phytochemicals and antioxidant properties. It has been used as a sweetener and contains no calories. Steviol and stevioside constitute the main parts of the chemical composition, an alternative to saccharose for the treatment of many diseases. This review article includes the collection of basic data on Stevia plant and the biochemical composition, nutrition and its therapeutic values, biochemistry and its uses in different fields and it's beneficial role and metabolites on properties that are health promoting.

Key words: **Stevia rebaudiana , Steviol, natural sweetener, therapeutic agent, phytochemicals.**

1 INTRODUCTION

Nature has bestowed us with an enormous wealth of medicinal plants, one of which is Stevia rebaudiana. They(medicinal plants) have been recognized as drug candidates. Stevia, a natural sweetener, produces medicinal plants having nutritional, industrial, and therapeutic importance used all over the world. The genus Stevia contains 250 species of herbaceous and shrubby plants, but up to 300 of them are members of the Asteraceae family. Though there are 250 species of the genus Stevia, the only species having the sweetest essence is S. rebaudiana. (Savita et al., 2004; Singh band Rao, 2005). Among these species, only Stevia rebaudiana and Stevia phlebophylla produce Steviol glycosides.

Stevia rebaudiana is native to Brazil and Paraguay. Today, Stevia has been cultivated in many parts of the world, like Canada and some areas of Europe. The first country to introduce Stevia as a natural sweetener in Asia for the drug and food industry was Japan. As compared to sucrose, it is 300 times sweeter. (Yadav et al., 2011). This review aims to promote the acceptance and better understanding of Stevia as a suitable raw material for the human diet.

2 Botanical description:

Stevia is a small perennial branched shruby plant which is 65-80 cm tall. This genus comprises about 110 species, but now 300 species are known. About 200 to 250 species of Stevia grow wildly, but Stevia rebaudiana and some other species have sweetening properties. It grows 65 to 80 cm tall with an extensive root system. It has a woody stem and a weak pubescent base. Leaves are sessile, elliptical, and 3–4 cm long, with blunt tips. Leaves are oppositely arranged. Flowers are white in color with a pale yellow. They are arranged in the form of corymbs and are smaller in size. (Goettemoeller and Ching, 1999; Singh and Rao, 2005).

3 Propagation:

Stevia can be propagated by using seeds or vegetative propagation. In the wild, reproduction is mainly by seed,but establishment and germination by seed are often poor and unsuccessful(Shaffert and Chebotar 1994). Due to poor seed production and low germination capacity, propagation through seed is uncommon. Propagation by stem cutting is quite easy, but requires high labor output. Cuttings of new

stems and shoots can be mostly propagated successfully(Lee et al., 1991; Shock, 1982; Gvasaliya et al., 1990; Nishiyama et al., 1991).

4 Taxonomical description:

Taxonomical information for *Stevia* rebaudiana is present in the following table 1:

Botanical name	*Stevia rebaudiana*
Kingdom	Plantae
Division	Angiosperm
Class	Eudicots
Order	Asterales
Family	Asteraceae
Genus	Stevia
Species	*Stevia rebaudiana*

Table 1: Taxonomic information of *Stevia rebaudiana*

5 Phytochemical constituents of stevia:

Plants accumulate secondary metabolites called phytochemicals. Many types of secondary metabolites are accumulated within the plant body. These metabolites defend the plant from any kind of infection or infestation caused by pests. Phytochemicals are the important ingredients having therapeutic properties. That is why they are used as a medicine or drug. In the solvent system of stevia, it also shows the presence of various phytochemicals. For example,

Phenols:
Phenols give anti-apoptotic, anti-inflammatory, anti-oxidant, and anti-aging properties to plants(Archana *et al.,* 2012).

Saponins:
These are anti-bacterial agents, surface active and foaming agents. They are used in detergents and are used to treat diabetes, hyperglycemia, and obesity. They are used as pesticides and molluscicides(Archana *et al.,* 2012; Mohanta *et al.,* 2007).

Flavonoids:
They are used in anti-allergy, anti-cancer, anti-microbial, and scavenging free radical activities. They are used to treat intestinal disorders and also prevent oxidative damage.(Archana *et al.,* 2012; Kam and Liew, 2002; Rio *et al.,* 1997; Salah *et al.,* 1995).

Alkaloids:
They are used in painkiller medication(Cushine and Lamb, 2006).

Tannins:
They have wound-healing properties. Also used in tissues affected by ulcers and in the treatment of diarrhea and dysentery(Rio *et al.,* 1997; Ranjan *et al.,* 2013).

Steroids:
They regulate the immune system and reduce hyper-cholesteolemic activities(Shah *et al.,* 2009).

Cardiac Glycosides:
They have Na+ and K+ pump inhibitory properties, which improve cardiac output and reduce heart distension(Schnieder and Wolfing, 2004).

Coumarins:
They prevent hyper- proliferative skin diseases. They have anti-bacterial and anti-inflammatory values(Theis and Lerdau, 2003).

Minerals:

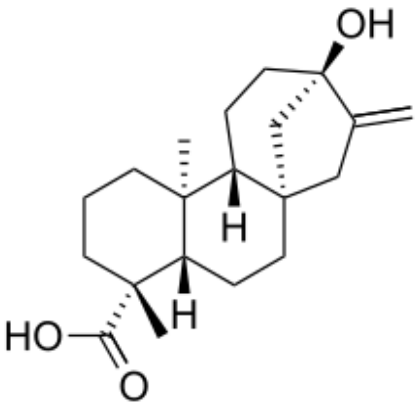
Minerals are also present in the dried leaf content of stevia. Minerals play an important role in health, reproduction, growth, and development of many cells and tissues in the body. They are also involved in various metabolic reactions within the body.

Stevia contains a variety of minerals, including tannins, alkaloids, glycosides, sterols, and triterpenes, according to phytochemistry.

5.1 Stevia as an alternative to artificial sugar (bio-sweetner):

The members of genus Stevia contain natural sweetening compounds, but Stevia rebaudiana is the sweetest among all species. It contains natural alternatives to artificial sweeteners . It contains over 100 phytochemicals. Stevia is enriched in many nutrients for example, it contains 80- 85 percent water, amino acids, fibers, lipids,some essential oils,free sugsrs,organic acids and some vitamins.It also contains some calcium, magnesium, iron and many other trace elements. Stevia rebaudiana contains an important component called Steviol glycosides. It is individually important because it has zero calorie value, a sweet taste, and an antidiabetic herb. As it contains zero calories, it is not absorbed by the human body. As it is absorbed, it is excreted through urine.

Steviol, a natural sweetening agent and an alternative to artificial sugar, is derived from the leaves of Stevia rebaudiana, a perennial herb. It acts as a backbone in the sweetening properties of Steviol glycoside and it is analyzed by the MS test.



Steviol, the basic building block of Stevia glycosides.

The most important compounds are steviol, which is made up of 0.9% stevioside, 2-5% rebaudioside, 4-13% glycoside, and 0.4-0.7% dulcoside. Steviol glycosides can be absorbed and eliminated through similar pathways in animals and humans. The most important sweetening agents present in stevia are glycoside and rebaudioside. The other sweetening agents are present in lesser quantities. The sweetening agents of Stevia constitute 14% of the dry leaves by weight. It contains 30 to 150 times the sweetness of sugar. In fact, it is 300 times sweeter than table sugar. It can tolerate heat, maintain pH, and can be fermented. The bodies of humans and animals are not able to metabolize the glycosides in stevia, so stevia doesn't contain any calories, just like many artificial sweeteners. The taste of stevia has a slower onset, but it has a long-lasting duration as compared to any other sugar, and some of its extracts may have a bitter after taste.

The main source of energy of Stevia is carbohydrates, mainly due to the presence of poly-oligosaccharides and fructo-oligosaccharides that enhance metabolism of lipids and therefore lower the blood sugar level. Stevia contains 1.9-4.3 kg/100 g of fat, 52-64.06 g of carbohydrates, and 10.0–18.0 g of proteins, respectively.

The importance of **stevia** as a food product changes from country to country. Since 2008, Stevia glycosides extracts have been generally recognized as safe to use in food items, but the leaves of Stevia and crude extract still not approved by GRAS and the food and drug administration for use in food products. In 2011, the European Union approved Stevia additives. In Brazil and Paraguay , stevia is used in local teas and medicines. In Japan, Stevia has been used as a sweetener for decades.

6 In vitro studies:

When *Stevia rebaudiana* bertoni is cultured on Murashige and on the Skoog medium, then it can regenerate the shoots from the shoot apex, nodal and leaf explant. Experiments were performed on the in vitro culture of *Stevia rebaudiana*.

Stevia cultured in the green house, shoot apex, nodal, and leaf explants for a young growing plant ranging in size from 1 to 1.2 cm have been collected. The plants from which the shoot apex, nodal and leaf explants were taken were cultured in the greenhouse having a temperature of 28 degrees Celsius and humidity of 50% . With the spray of 0.5%(w/v) bavistin, they were watered twice a day for a week. After this ,they were supplied with tap water for 20 minutes and immersed in the 5% (v/v) bacillocid and 0.25% w/v bavistin for 10 minutes. Further sterilization has been carried out in the laminar airflow chamber after 3 washes with double distilled water. The chamber contained 0.1%(w/v) mercuric chloride for 10 minutes. The plants were then rinsed with sterile water three times. Inoculation of stevia plants was carried out on Muraushig and Skoog media supplemented with cytokines and auxin. The pH of the MH media was adjusted to 5-8. Before autoclaving, 0.7% of agar was added. In their combined form, the nutrients are used for shoot induction and the best result was obtained from MS medium supplemented with Benzyl adenine + Indole Acetic acid at concentrations of 1.0 mg/l and 0.5 mg/l. If MS medium doesn't contain any growth regulator, then it doesn't promote the growth of shoots.

A 2 to 2.2cm shoot was subcultured on agar gelled medium and MS media supplied with insole-3-butyric acid . It occurred after 40 days. Deflasking was done after 5 days. Now the plantlet was transferred into a 6 cm nursery pot containing soil ,coco peat, and vermiculite. It was used singly and in combination.

The sweetening principles, viz. stevioside and rebaudioside, appeared as a grey zone on the visual observation of HPTLC (high performance thin layer chromatography) plates. On scanning plates at 200nm and standard, the samples gave peaks having Rf values in the range of 10–0.45. The presence of active leaves was confirmed by the in vivo and in vitro derived leaves of the stevia plant. These included NAA (Naphthalene Acetic acid) among auxins and BA (6-benzyl adenine) among cytokines.

In vitro studies of Stevioside show that it is not digested by the human body. The human body doesn't have any enzymes to digest the Stevioside. It is hydrolyzed by the bacteria in the colon into Steviol, Steviol 16, and 17alpha epoxide. The Steviol 16 and 17alpha epoxide are again converted into Steviol, which is then excreted through urine in the form of Steviol glucuronide.

6.1 Seed germination and cultivation:

Seed germination is the first phase of plant growth and development. Two hormones play an important role in germination. One is abscisic acid (ABA) and the other is gibberellic acid (GA). Their actions are antagonistic to each other. Gibberellic acid promotes seed germination while abscisic acid inhibits seed germination and promotes seed dormancy. Seed germination influences crop yield and quality. An inverse relationship generally exists between the

percentage of seed germination and time of seed germination and level of salinity. Salinity involves many changes in systematic functioning and metabolic changes such as potassium efflux, solute leakage, and alpha-amylase.

7 Therapeutic values of Stevia:

Stevia rebaudiana has been used since ancient times for the treatment of various diseases. Its leaves play an important role in curing various diseases. They have antioxidant, anti-microbial, anti-inflammatory, and anti-tumor properties. anti-carcinogenic and antifungal applications. It is used to cure diabetes mellitus. Cardiovascular diseases, cancer, renal diseases, obesity, inflammatory bowel disease, diarrhea, etc . During the treatment of diabetes, the leaf extract of Stevia after ingestion causes an increase in glucose forbearance and also slightly suppresses plasma glucose level. Stevioside and Steviol are important glycemic agents. Steviol glycoside acts on beta cells and thus increases insulin secretion without affecting the activity of potassium-ATP channels or cAMP levels in islets. The use of stevia has reduced the risk of cancer and many heart diseases. It also inhibits tumor intition and proliferation. Stevia is also used in the treatment of renal and bowel diseases. Its bacteriostatic and bacteriosidal properties avoid the risk of tooth decay and gingivitis. As Stevia has no calories, it helps in weight loss as compared to table sugar. So, due to its property of weight loss, it is gaining importance commercially. Stevioside and other extracts from stevia are nephro-protective as they have coinciding activities like antioxidative potential, inflammation, and apoptosis. The aqueous medium of Stevia controls the cholesterol level in the blood. Therefore, Stevia also acts as a hypo-lipidemic substance . The inhibition of oxidative stress by stevia can be utilized for the treatment of liver injury like hepatic carcinoma and cirrhosis, which is caused by oxidative potential. Stevia provides 2.7 kcal/g of energy. It has no cancerous and non-allergic effects when it is used as a sweetener. Stevia has anti-fungal and anti-bacterial activity.

7.1 Stevia as an antioxidant:

Stevia rebaudiana as protein, lipids, nucleic acids, and carbohydrates. When conflict occurs between the release of reactive oxygen species and the ability of the human body to spontaneously deoxify free radicals to repair the subsequent deficiency, then this disproportion or conflict leads to oxidative stress. The anti-oxidant activity was verified by the diphenyl 1–picryl hydrazyl hydrate, radical scavenging test, FRAP (ferric acid reducing activity) test, and phospho molebednum test while in vitro conditions. Both methanoic and aqrous extracts of the dry form of stevia leaves contain high concentrations of polyphenols like hesperidin, chlorgenic acid, eugenol, coumarin, vanillin, and flavonoids, and thus they can be used as a beneficial source in food and beverages and as important ingredients for diseases like diabetes, cancer, neural disorders, aging, and arthritis, which is a disease caused by the production of ROS (reactive oxygen species). This potential of stevia as an antioxidant has the ability to beat the synthetic antioxidants like BHA(butylated hydrocyanic) and BHT(butylated hydroxytoulene), but it is now limited in use due to its cancer-causing properties. Hydrogen peroxide causes lipid peroxidation, which is a prominent reactive oxygen species and DNA damage in a cell. The generation. Reactive oxygen species are inhibited by the action of some natural compounds such as minerals, vitamins , polyphenols and some non-nutrient compounds of plants. Natural antioxidants have demonstrated a wide range of pharmacological activities, including anti-inflammatory, anti-cancer, and anti-aging properties. Synthetic antioxidants are also found, like Butylhydroxyanisole and Butyl hydroxy toluene. The Stevia plant and the extracts obtained from it are mainly studied due to their sweetening properties.

8 Uses of Stevia:

Stevia is a natural sweetener plant that is grown commercially in many parts of Brazil, Korea, China, and India, etc. It increases the quality of flavor, which gives help in digestion and weight loss. Anti-oxidants also prevent tooth decay due to antimicrobial and anti-plaque characteristics that increase mental alertness. It increases the energy of the body but can't affect the sugar level of the blood because they play a significant role in the diabetic world. It is used to treat hypertension, lower blood sugar levels, and clean oil from the skin. It is also used to treat tobacco and as a pancreas tonic. It is used as a substitute for sugar for food industries, bakeries, fruit jams, juices, biscuits, vegetables, chocolates, and other foodstuffs. Their leaves are the most abundant source of sweet glycosides, and the ratio of steriosides in leaves is higher when the stevia plants are grown under long days and harvested just before flowering. Stevia is a small shruby plant that has been used for many years as a natural sweetening agent and it also has some medicinal properties. They are used sparingly, and there seems to be no threat to public health. Steviol glycoside is useful for obesity, diabetes, and heart disease. Stevia helps in weight loss to satisfy sugar cravings. It helps to minimize blood sugar levels as an alternative. The leaves of Stevia have been used as herbal remedies in South America for several decades. Stevia extracts show anti-microbial activity against pathogenic bacteria. Steviol glycosides found in Stevia leaves have been shown in recent studies to have important anti-carcinogen activities against human gastrointestinal cancer cells. Steviol glycosides improved cardiovascular function and demonstrated hypertensive properties. Steviol glycoside extract is reported to increase sodium excretion , urine output, and dilation of blood vessels. Leaves of Stevia are commonly used in herbal teas and food, but they are most commonly used in beverages than in food. Stevia is used as a tonic to decrease depression. Some phenolic compounds such as Stevioside are present in Stevia. They protect plants from infection by bacteria and microorganisms in case of injuries. Although these compounds don't have any nutritional importance for

humans, they have antioxidative properties that are beneficial for humans in various aspects.

9 Improvements:

Stevia rebaudiana is an important plant which has been used for the production of non-nutritive, non-toxic, high-potential sweeteners. It has many important properties as it is used to stop the growth of many bacteria and fungi, as well as cancerous cells. It is also an anti-hyperglycemic and anti-hypersensitive agent. It protects the teeth from decay.

The *Stevia rebaudiana* crop has many improved features, such as development of new varieties, large scale production and transmission of healthy characteristics to offspring, seed production, cultivation, disease prevention, improvement of the glycoside quality and quantity. If Stevia plants are cultivated in large numbers, then the major problem is that the plants lack quality material. Then stevia is a that promotes outcrossing and plants grown by seeds show different growth patterns. Plants having preferable characteristics are propagated by tissue culturing, which prevents the undesirable production of planting materials such as pollen etc.

10 Conclusion:

Stevia rebaudiana is extensively used worldwide in the drug and food industries. During extraction, many polar compounds like chlorophyll, carotenoids, phenolic compounds are obtained. These compounds contribute to the antioxidative action of Stevia. The leaves of *S. rebaudiana* Bertoni could be used not only as a source of natural zero-caloric sweeteners but also as a naturally occurring antioxidant. Scientists are working to discover more therapeutical benefits of this plant. But still, its production quality is not standardized to meet its use in the food industry.

11 Acknowledgement:

The authors acknowledge the efforts of the Principal of Government Viqar-un-Nisa Post Graduate College, Rawalpindi ; Dr. Zahida Parveen, Head of Zoology Department, Dr. Mehmoona Naz, and Dean of Sciences, Mrs. Huma Haider.

References:

- Oxforddictionaries.com. British & World English. 7 February 2013. Archived the original on 12 February 2013. Retrieved 13 February 2013.
- Stevia Oxforddictionaries.com. US English. 7 February 2013. Archived from the original on 9 May 2013. Retrieved 13 February2013.
- " Is stevia a good sugar substitute" Journals. 19 January 2021. Retrieved 27 January 2021.
- Cardello, H.M.A.B.; da Silva, M.A.P.A.; Damasio, M.H. (1999). "Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations". Plant Foods for Human Nutrition. **54** (2): 119–129.
- ^ Brandle, Jim (19 August 2004). FAQ – Stevia, Nature's Natural Lowrie Sweetener (Report). Agriculture and Agri-Food Canada.
- " Has stevia been approved by FDA to use as a sweetner" US Food and Drug Administration. 28 April 2017. Archived from the original on 29 July 2017. Retrieved 27 July 2017.
- Stones, Mike (2011)." Stevia wins final EU approval" foodmanufacture.co.uk. Archived from the original on 18 November 2011. Retrieved 22 November 2011.
- Amzad Hossain et Al 2010.
- Acunal..NepovimA..and Valicek P 1997Micropropagation of plants of stevia rebaudiana in vitro and content of stevioside in leaves after application of growth regulators under field condition Agric.
- Parveen k. Kathore Module in life sciences, 2020.
- Pham Anh Tuan, Belay T. Ayele in sprouted Gr. 2019.
- Muhammad Riaz Advances in rice reserch for Abiotic stress tolerance, 2019
- MF Hossain, MT Islam , MA Islam, S A Akhtar – African Journal of Food, Agriculture, Nutrition and Development 2017(4).
- V Suresh, J Preethi Fetricia, V Saranya, S Sarithra, K Tamilselvan- J.Med Plant,2018.
- Carakostas MC, Curry LL, Boileau AC, Brusick DJ. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food Chem. Toxicol. 2008; 46: 1-10.
- Singh A, Agrawal N and Gupta AK. 'Sweet shrub' Stevia: More than a sweetner. Indian Journal of Drugs. 2014; 2: 1-4.
- Gupta E, Purwar S, Sundaram S and Rai GK. Nutritional and therapeutic values of Stevia rebaudiana: A review. Journal of Medicinal Plants Research. 2013; 7: 3343-3353.
- Lemus-Mondaca R, Vega-Gálvez A, Zura-Bravo L and Ah-Hen K. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chemistry. 2012; 132: 1121-1132.

- McChesney JD. Stevia: The Genus Stevia, edited by A. Douglas Kinghorn (University of Illinois at Chicago). Taylor & Francis, London. 2006.
- Chabot S. Improved stevia rebaudiana extract and formulation, and uses thereof. 2013.

- Barba, Francisco J., Predrag Putnik, and Danijela Bursac Kovacevic, eds. Agri-Food Industry Strategies for Healthy Diets and Sustainability: New Challenges in Nutrition and Public Health. Academic Press, 2020.
- Díaz-Gutiérrez, Carol, et al. "Increase in steviol glycosides production from Stevia rebaudiana Bertoni under organo-mineral fertilization." Industrial Crops and Products 147 (2020): 112220.
- Nguyen, Thi Thanh Hanh, et al. "Production of steviol from steviol glucosides using β-glucosidase from a commercial pectinase, Sumizyme PX." Biotechnology letters 40.1 (2018): 197-204.
- Ahmad, Jamil, et al. "Stevia rebaudiana Bertoni.: an updated review of its health benefits, industrial applications and safety." Trends in Food Science & Technology (2020).
- Hossain, M. F., et al. "Cultivation and uses of stevia (Stevia rebaudiana Bertoni): A review." African Journal of Food, Agriculture, Nutrition and Development 17.4 (2017): 12745-12757.
- Kovačević, Danijela Bu sać, et al. "Innovative technologies fo the ecove y of phytochemicals from Stevia rebaudiana Bertoni leaves: A review." Food chemistry 268 (2018): 513-521.
- Hutchings, Scott C., Julia YQ Low, and Russell SJ Keast. "Sugar reduction without compromising sensory perception. An impossible dream?." Critical reviews in food science and nutrition 59.14 (2019): 2287-2307.
- Kim, Byeong Jo, Sangwook Lee, and Hyun Suk Jung. "Recent progressive efforts in perovskite solar cells toward commercialization." Journal of Materials Chemistry A 6.26 (2018): 12215-12236.
- Ortiz-Viedma, Jaime, et al. "Anti-oxidant and antimicrobial effects of stevia (Stevia rebaudiana Bert.) extracts during preservation of refrigerated salmon paste." European Journal of Lipid Science and Technology 119.10 (2017): 1600467.
- Gao, Yu-Qi, et al. "Microbial Transformations of Two Beyerane-Type Diterpenes by Cunninghamella echinulata." Journal of Agricultural and Food Chemistry 68.16 (2020): 4624-4631.
- Anker, Camilla Christine Bundgaard, Shamaila Rafiq, and Per Bendix Jeppesen. "Effect of steviol glycosides on human health with emphasis on type 2 diabetic biomarkers: A systematic review and meta-analysis of randomized controlled trials." Nutrients 11.9 (2019): 1965.
- Lemus-Mondaca, Roberto, et al. "Anti-oxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods." Journal of Applied Research on Medicinal and Aromatic Plants 11 (2018): 37-46.
- Pallepati, Akhil, P. Yavagal, and D. J. Veeresh. "Effect of consuming tea with stevia on salivary ph-an in vivo randomized controlled trial." Oral Health Prev Dent 15.4 (2017): 315-319.
- Cocco, Fabio, et al. "Effect of a daily dose of snacks containing maltitol or Stevia rebaudiana as sweeteners in high caries risk schoolchildren. A double-blind RCT study." Oral Health Prev Dent 17 (2019): 515-522.
- Adesh AB, Gopalakrishna B, AkkiKusum S, Tiwari OP (2012) An over-view on stevia: a natural calorie free sweetener. Int J Adv Pharma Biol Che 1: 362-368.
- Yadav AK, Singh S, Dhyani D, Ahuja PS (2011) A review on the improve-Ment of stevia [Stevia rebaudiana (Bertoni)]. Can J Plant Sci 91: 127-130.
- Braz-De-Oliveira A, Goncalves RAC, Chierrito TPC, Souza L, Gorin PAJ, Et al. (2011) Structure and degree of polymerisation of fructooligosac-Charides present in roots and leaves of stevia rebaudiana bertoni. Food Chem 129(2): 305-311.
- Cacciola F, Delmontea P, Jaworska K, Dugo P, Mondello L, et al. (2011) Employing ultra-high pressure liquid chromatography as the second Dimension in a comprehensive two-dimensional system for analysis of Stevia rebaudiana extracts. J Chromat 1218: 2012-2018.
- Raut D, Aruna K (2017) Antimicrobial activity of stevia rebaudiana Against antibiotic resistant ESBL producing uropathogens and evaluation of its antioxidant activity. Int J Adv Res Biol Sci 4: 110-118.
- Miranda-Arambula M, Olvera-Alvarado M, LoboSanchez M, Perez-Xo-Chipa I, Rios-Cortes AM (2017) Antibacterial activity of extracts of steVia rebaudiana bertoni against staphylococcus aureus, staphylococcus Epidermidis and

pseudomonas aeruginosa. J Med Plant Res 11: 414-418.

- Goyal S, Samsher R (2010) Stevia a bio-sweetener: a review. Int J Food Sci Nutr 61: 1-10.
- López-Carbón V, Sayago A, González-Domínguez R, Fernández-Recamales Á (2019) Simple and efficient green extraction of steviol glycoSides from stevia rebaudiana leaves. Food 8(9): 402-406.
- Kroyer G (2010) Stevioside and stevia-sweetener in food, application, Stability and interaction with food ingredients. J verb lebensm 5: 225-229.
- Marcinek K, Krejpcio Z (2015) Stevia rebaudiana bertoni–chemical Composition and functional properties. Acta Sci Pol Technol Aliment 14(2): 145-152.
- Shakya AK (2016) Medicinal plants: future source of new drugs. Int J Herbl Med 4: 59-64.
- Srivastava S, Chouhan DES, Chawla P (2016) Proximate and phytochemical analysis of stevia leaves powder. Int Ayurved med J 4: 2476-2480.
- Gasmalla MAA, Yang R, Issoufou A, Hua X (2014) Nutritional composition of stevia rebaudiana bertoni leaf: effect of drying method. Trop J Pharm Res 13: 61-65.
- Kobus-Moryson M, Gramza-Michalowska A, Kobus-Cisowska J, Korczak J (2014) Contents of selected elements in extracts from sweet leaf (stevia rebaudiana Bertoni). Probl Hig Epidemiol 95: 445-448.
- Ahmed SR, Mukta S (2017) Review on stevia rebaudiana as a non-caloric natural sweetener producing plant. J Syl Agric Uni 4: 15-25.
- Shuvo MMA, Al–Mamun M, Chowdhury T, Absar N, Hasanuzzaman M (2015) An assessment of major nutritional components and some secondary metabolites of in vitro propagated stevia rebaudiana (cultured In Bangladesh) plant leaves dry powder. Int J Applied Sci Biotechnol 3: 721-26.
- Gupta E, Purwar S, Sundaram S, Rai GK (2013) Nutritional and thera-Peutic values of stevia rebaudiana: a review. J Med Plant Res 7: 3343-53.
- Escutia-López KN, Sánchez-Pardo ME (2019) A comprehensive review On the nutritional and therapeutical aspects of stevia rebaudiana bertoni. J Appl Biotechnol Bioeng 6: 297-302.
- Salehi B, López MD, Martínez-López S, Victoriano M, Sharifi-Rad J, et al. (2019) Stevia rebaudiana bertoni bioactive effects: from in vivo to clinical trials towards future therapeutic approaches. Phytotherap Res 33(11): 2904-2917.
- Shannon M, Rehfeld A, Frizzell C (2016) In vitro bioassay investigations of the endocrine-disrupting potential of steviol glycosides and their metabolite steviol, components of the natural sweetener stevia. Mol cell endocrinol 427: 65-72.
- Singh DP, Kumari M, Prakash HG, Rao GP, Solomon S (2019) Phytochemical and pharmacological importance of stevia: a calorie-free natural sweetener. Sugar Tech 21: 227-34.
- Ahmad U, Ahmad RS (2018) Antidiabetic property of aqueous extract of stevia rebaudiana bertoni leaves in streptozotocin-induced diabetes in albino rats. BMC Compl Alter med 18: 179-182.
- Scaria A, Kamath JV, Chakraborty M (2017) Antihyperglycemic, Antioxidant, Anti hyperlipidemic & nephroprotective effect of stevioside in diabetic rats. Int J Ayurved Med 8: 178-813.
- Ilić V, Vukmirović, Tilinović N, Čapo I, Arsenović M, Mili ašević B (2017) Insight into anti-diabetic effect of low dose of stevioside. Biomed Pharmaco 90: 216-221.
- Prata C, Zambonin L, Rizzo B, Maraldi T, Angeloni C, et al. (2017) Glycosides from stevia rebaudiana bertoni possess insulin-mimetic and antioxidant activities in rat cardiac fibroblasts. Oxidat med cell longev 11: 140-148.
- Aghajanyan A, Movsisyan Z, Trchounian A (2017) Antihyperglycemic and antihyperlipidemic activity of hydroponic stevia rebaudiana aqueous extract in hyperglycemia induced by immobilization stress in rabbits. BioMed Res int 10: 120-123.
- Assaei R, Mokarram P, Dastghaib S, Darbandi S, Darbandi M, et al. (2016) Hypoglycemic effect of aquatic extract of stevia in pancreas of diabetic rats: PPARγ-dependent regulation or antioxidant potential. Avi J Med Biotechnol 8: 65-69.
- Bayat E, Dastgheib S, Egdar S, Mokarram P (2017) Effect of the aquatic extract of stevia on the serum level of interleukin-6 in streptozotocin-nicotinamide induced diabetic rats. Shiraz E-Med J 18: 160-170.
- Chavushyan VA, Simonyan KV, Simonyan RM, Isoyan AS, Simonyan GM, et al. (2017) Effects of stevia on synaptic plasticity and NADPH oxidase level of CNS in conditions of metabolic disorders caused by fructose. BMC Compl Alter Med 17(1): 540-543.
- Najafian S, Moradi M (2017) Polyphenolic compounds (HPLC analysis) and antioxidant activity of stevia rebaudiana (Asteraceae) by FRAP and DPPH assay in greenhouse and free space condition. Int J Farm Allied Sci 6: 49-55.

- Mohammad AM (2018) In vitro determination of total phenolics, flavonoids and free radical scavenging activities of stevia rebaudiana dry leaves powder in different solvents extract. EC Nutr 13: 71-78.
- Yücesan B, Gurel E (2018) Hydrogen peroxide-induced steviol glycosides accumulation and enhancement of antioxidant activities in leaf tissues of stevia rebaudiana bertoni. Sugar Tech 20: 100-104.
- Ruiz-Ruiz JC, Moguel-Ordoñez YB, Matus-Basto AJ, SeguraCampos MR (2015) Antidiabetic and antioxidant activity of stevia rebaudiana extracts and their incorporation into a potential functional bread. J Food Sci Technol 52: 7894-7903.
- Li XY, Lu WM, Shen WF, Wu Y, Liu YP, et al. (2017) Growth inhibitary effect of stevioside on ovarian cancer through AKT/ERK pathway. Biomed Res 28: 1820-1827.
- Potocnjak I, Broznic D, Kindl M, Kropek M, Vladimir Knezevic S, et al. (2017) Stevia and stevioside protect against cisplatin nephrotoxicity through inhibition of ERK1/2, STAT3, and NF-kB activation. Food Chem Toxicol 107: 215-225.
- Hagh-Nazari L, Goodarzi N, Zangeneh MM, Zangeneh A, Tahvilian R, et Al. (2017) Stereological study of kidney in streptozotocin-induced diabetic mice treated with ethanolic extract of Stevia rebaudiana (bitter Fraction). Comp Clinic Pathol 26: 455-463.
- Wang Y, Li L, Wang Y, Zhu X, Jiang M, Song E, Song Y (2018) New application of the commercial sweetener rebaudioside as a hepatoprotective candidate: induction of the Nrf2 signaling pathway. Europ J Pharmacol 8: 128-137.
- Latha S, Chaudhary S, Ray RS (2017) Hydroalcoholic extract of stevia Rebaudiana bertoni leaves and stevioside ameliorates lipopolysaccharide-induced acute liver injury in rats. Biomed Pharmaco 95: 1040-1050..
- Arya A, Kumar S, Kasana MS (2012) In vitro regeneration of stevia and Evaluation of antimicrobial and antiprotozoal properties of regenerated calli and plants. Elect J Plant Breed 3: 916-924.
- Carreras MC,Franco MS,PeraltaJC nitric oxide complex 1 and the modulation of mitochondrial reactive species in biology and disease ; Mol Asp Med2004:25;125_39.
- Saradha M,Paulsamy S, In vitro antioxidant activity and polyphenol estimation methanolic extract endagered medicinal species, Hildigardia populifolia (Roxb) , Int J phytomed 2012 ;4 362-368.
- Ebrahimzadhe MA ,Nabvi SF,Nabavi SM Salami B,Antihemolytic and Antioxidant activity Allum paradoxum .Cent Ent J Biol 2010,5;338-345.
- Rao GN ,Rao PP,Balaswmy k, Antioxidant activity of stevia , leaf powder and a commercial stevioside powder, J food pharms Sci 2014 ,2:32-38.
- C bender,S Graziano international journel 2015 ,Taylor and Francis.
- Felippe, G. M.; Lucas, N. M. C.; Behar, L.; Oliveira, M. A. C. Observacoes a Respeito de germinacao de Stevia rebaudiana Bert. Hoehnea. 1:81–93; 1971.
- Ferreira, C. M.; Handro, W. Micropropagation of Stevia rebaudiana through Leaf explants from adult plants. Plant Med. 54:157–160; 1988.
- Ishima, N.; Katayama, O. Sensory evaluation of stevioside as a sweetener. Rep.
- Natl Food Resp. Inst. 31:80–85; 1976.
- Pól, J., Hohnová, B., & Hyötyläinen, T. (2007). Characterization of Stevia rebaudiana by comprehensive two-dimensional liquid chromatography time-of-flight mass Spectrometry. Journal of Chromatography A, 1150, 85–92.
- Chatsudthipong, V., & Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacology & Therapeutics, 121,41–54.
- Goyal, S., Samsher & Goyal, R. (2010). Stevia (Stevia rebaudiana) a bio-sweetener: A review. International Journal of Food Sciences and Nutrition, 61, 1–10.