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MORPHOLOGY, CHEMICAL COMPOSITION AND THERAPEUTIC POTENTIAL OF *STEVIA REBAUDIANA*

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Abstract:
Sugar related disorders are significant causes of global death among all age groups. The use of artificial sweetener as a sugar supplement is a common practices, but causes side effects especially on weight gain and heart related problems. The use of natural sweetener obtained from Stevia rebaudiana leaves has been practiced since 18th century and has gained more popularity in last two decades. The natural sugar supplement is of low calorie hence safe to be consumed. The chemical steviol glycosides mainly present in form of stevioside and rebaudioside in the leaves makes it commercialy significant as a natural sugar substitute. Besides it also contain nutrients, vitamins, fiber, volatile oil and thus thirty times sweeter than white sugar. The leaves also possess antimicrobial, antifungal, antidiabetic, anticariogenic, antioxidant, hypotensive, anti-inflammatory, antihypertensive and antitumor activities. The aim of this article is to project the economic value of S. rebaudiana *leaves as natural sweetner and its potentiality as health-product. The study emphasize to enhance quality in leave composition using new genetic engineering and other biotechnological techiques with sustainable use and preservation.*

Keywords: *Stevia rebaudiana, natural sweetener, chemical composition, medicinal use*

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INTRODUCTION:

Sugar disease is a matter of global concern leading to almost half of all deaths and according to WHO the disease will be placed number seventh among all the global death causes upto 2030 [1]. The disease manifests in three different ways- low blood sugar (Hypoglycemia), excess fat and cholesterol and plentiful carbohydrates (Syndrome X), and failure(Diabetes type 1) or overproduction of insulin (Diabetes type 2)[2]. Treatment of sugar diseases using high-intensity artificial sweeteners like sucralose, saccharin and aspartame is common, but also resulted in increased risk of metabolic syndrome, weight gain, type 2 diabetes, and cardiovascular disease [3].In recent years the use of naturopathy to introduce innovative and novel bioactive compounds from natural products has increased globally[4] and has given a hope to introduce an alternative natural sweetener to treat sugar disorders.

Stevia rebaudiana sp. Bertoni commonly referred as "Sweet leaf" or "Sugar leaf", is an important sugar substitute plant recognized throughout the globe. Earlier the plant acceptability as a natural sweetener was a matter of controversy as in some countries like Japan and Korea the refined leaf extracts are used to substitute for sucrose while the ban on the import of the plant in 1991 and again its removal in 1995 by the US Food and Drug Administration and imposing restriction on all products prepared from *S. rebaudiana* leaves or their constituents by UK government raising question on its safe use, has created lots of confusion about its safe use [5]. Finally, after a prolonged study and research and immense global response, the USFDA has approved high-purity leaf extracts as safer natural, non-nutritive sweetener (NNS) along with monk fruit (Siraitia grosvenorii Swingle) and six other artificial NNS as food additives [6]. The main cause of depletion of useful plant population is mainly due to extraction of chief constituent from these plant which lead them under legal restrictions[7].

Presently, Stevia as a major source of high-potency sweetener has become a subject of research challenging researcher to develop a crop that should be well suited under changing agricultural conditions. Understanding the biology, chemistry and biochemistry of the sweet component are prerequisites that can be made possible by developing quality seed, plantlets and crop production involving information on optimized growth conditions, combat various stresses, harvest and a breeding program on chemical contents and sensory nature of the plant [8]. There are various work based on introducing innovative and discovering novel bioactive compounds of natural origin [9,10]. Various researches are conducted on chemical composition and uses of valuable plants present in the nature and their conservation [11], and the need for preparing natural products to fight chronic diseases has become more demanding[12].

History

The use of a leaf extract as sweetening food additive started long back in South America [13], followed by the European Union and the USA. The available historical documents suggest that the Guarani Indians of Amambay Mountain in Rio Monday valley in Paraguay use a "sweet herb" who called them 'kaa he-e' which was later introduced from old world to a new world in the 1800s, finally gains its popularity in other South American countries [14]. The genus 'Stevia' derived its name in the 16th century in honor of the Spanish botanist and physician Pedro Jaime Esteve [15]. The discovery of new world Stevia credited to a Paraguayan Agriculturist, Moises Santiago Bertoni from Paraguay forest in 1887, and species name 'Rebaudi' in honor of a chemist who for the first time extracted the 'sweet constituent', suggesting that the plant has power superior to sugar and has great economic value [14]. Stevia leaf extract was first commercially adopted by Japan in the 1970s [5,16]. At present, the plant is commercially cultivated in various countries including Paraguay, Argentina, Germany, China, UK, Japan, Spain, South Korea, Canada, Australia, Mexico, Belgium, United States, Brazil, Israel, Malaysia, Indonesia, Taiwan, Thailand, Tanzania and India [17-22].

Description and distribution

Stevia rebaudiana a member of the family Asteraceae is a small perennial semi-humid shrub attaining a height up to 30 cm [23] or even 65 cm and at maturity 80cm, having upright woody

Fig.1: Stevia leaves

stems with sessile and opposite lanceolate to oblanceolate leaves. Leaf surfaces slightly glandular having two distinct sizes of the trichome. The inflorescence is a chyme of corymbs containing five small white tubular flowers. The fruit is an achene,

having a single seed with a feathery pappus. The plant grows best in day temperature 20-28^{0}C and night temperature 13-20^{0}C with 80% relative humidity [8,24-26]. The plant requires well-drained sandy loam or red soil with pH 6.5- 7.5 [27,28]. Stevia is an obligate short-day and diploid with 11 chromosome pairs [29] and can be propagated from cuttings or seed. Economically, plant production through transplantation produced from seed is the best method to raise the crop on a large scale [8].

CHEMICAL COMPOSITION:

Various studies has been conducted to study the phytochemistry of *Stevia rebaudiana*. Phytochemical analysis of the leaves shows the presence of alkaloids and steroids in abundance along with tannins, saponins, flavonoids, glycoside, sterol, triterpenes, anthraquinones, reducing compounds, vitamin C, folic acid, all of the indispensable amino acids, nonglycosidic diterpenes, chlorogenic acids, nutrients, vitamins, and other minor compounds [30-33]. Gas chromatography analysis reveals the presence of palmitic, stearic, palmitoleic, oleic, linolenic and linoleic acids in leaf oil [31].Mineral analysis of plant leaves using atomic absorption spectrophotometry reveals high content of potassium, phosphorus, calcium, magnesium, sulphur and sodium and a trace amount of iron, copper, cobalt, manganese, zinc, selenium and molybdenum. The estimation of leaf oil applying standard methods show high amounts of carbohydrate, followed by ash and protein and a low amount of fat on dry weight basis [31]. The leaves also contain 2.857% foreign matter, 2.903% w/w total ash, 9.4127% moisture content with 27.278% aqueous soluble and 30.573 % w/w alcohol soluble solvent extractive values [30].

The sweet principles in the Stevia leaves are mainly due to the presence of eleven steviol glycosides, most importantly being stevioside and rebaudioside A [16,32,34,35]. Pure leaf extract contains Stevioside which is 150-300, Rebaudiosides A 200-400, Rebaudioside B 300-350, Rebaudioside C 50-120, Rebaudioside D 200-300, Rebaudioside E 250-300, Rubusoside 110, Steviolbioside 100-125 and Dulcoside A 50-120 times sweeter than sucrose, whereas Rebaudioside F, Steviolmonoside composition is not clearly known [16]. Isocratic HPLC methods have also detected minor glycosides like steviolbioside, dulcoside A, rebaudioside B and rebaudioside C from the leaves [36].Another non-caloric compound Rebaudioside E is also present in a minor amount in the leaves and about 150-200 times sweeter than common sucrose. NMR spectroscopy reveal that rebaudioside E has a nominal mass of 966 Daltons with the molecular formula $C_{44}H_{70}O_{23}$ and is, therefore, an isomer of rebaudioside A (3) [37].

The maximum amount of steviol glycosides is present in leaves followed by flowers, stems, seeds, and roots. The mature leaves and stems possess more glycosides which continue up to bud phase and preliminary flowering stage [35]. The steviol glycosides (SG) are thermostable even at temperatures up to 200^{0}C and are used in cooked foods [32]. Stevia leaf available commercially in the form of the stevioside powder which is hygroscopic with 24% relative humidity containing a high amount of polyphenol contents [38]. One of the main problems in the successful commercialization of plant sweetener is its slight acrid caustic aftertaste which may be reduced by altering carbohydrate moieties of steviol glycosides through enzymatic glycosylation and adding an additional monosaccharide residue in the molecules [39].

Various experiments were conducted on the interaction and functioning of stevioside and rebaudioside the chief content of this natural sweetener with internal human microbial activity. Fecal bacteria of human microbial fecal community, mainly the Bacteroides under anaerobic conditions, hydrolyzes stevioside and rebaudioside to Anglican steviol, which was not degraded by human intestinal bacteria [34]. There are the numerous studies that emphasize the importance of extraction method for the classification of medicinal plants from different cultivation conditions. The chromatography chemical fingerprint and pattern recognition tools including pressurized hot water extraction (PHWE) and microwave-assisted extraction (MAE) reveals that MAE was found more efficient than PHWE in providing distinctive chemical fingerprints for quality control purposes [40]. Certain techniques have also been tested to preserve the glycosides in dry leaves so as to select quality diterpene glycosides contents. In one such study the extraction of solvent followed by isocratic HPLC analysis was carried out at pH 5 and detected using the UV range at 210 NM and on the quantification of standard solutions of stevioside and rebaudioside A using external standard calibration curve reveals that the stevioside and rebaudioside A fractions were found in the range between 3.78 and 9.75 and 1.62 and 7.27% by weight respectively [41].

MEDICINAL USES:

The plants in the surrounding provides a valuable source of information to the users, however their proper conservation should be given priorities [42]. Various studies on stevia leaves supported its

antimicrobial, anticariogenic, antifungal, antioxidant antidiabetic, hypotensive, anti-inflammatory, antihypertensive and antitumor activities [32,33,38,43]. Agar-well diffusion method for inhibition activity against bacteria Streptococcus mutants reveal that at 100 mg/ml concentration acetone, followed by ethanol and methanol leaf extract shows inhibition 28.7, 27.0 and 21.3mm respectively [44]. The hexane, ethanol, methanol, ethyl acetate and chloroform leaf extract on 16 bacterial strains reported that the hexane extract at 30 mg/ml shows lowest MIC for 12 *Streptococcus* strains, whereas inhibition zone of the 5 leaf extracts was higher for 4 *Lactobacillus* strains proving that they were the most susceptible bacterial strains [45]. Water, alcohol, soxlhet and column leaf extract of the plant on certain bacterial inhibition reveals that *Enterobacter aerogenes* exhibited highest rate of susceptibility and other bacteria *Klebsiella aerogenes, Staphylococcus albus, E. coli* and *Bacillus subtiles* depicted considerable inhibition in first three extact. Among three tested fungi *Candida albicans* shows miimum zone of inhibition in Soxhlet leaf extract and other fungi *Penicillium chrysogenum* and *Aspergillus niger* exhibited higher inhibitions during the first 24 to 48 hrs and afterwards they started sporulation and do not shows inhibition zone in the tested extracts [30].

There are various researches on antioxidant activities of stevia leaves. Methanolic leaves extract using 1-diphenyl-2-picrylhydrazyl (DPPH) shows radical scavenging ability very much equivalent to gallic acid, a strong antioxidant chemical reagent [46]. The methanolic extracts of leaf powder have more DPPH radical scavenging activity and at 20 and 100 µg/ml concentration shows 30.33% and 52.46% inhibition compared to commercial stevioside [38]. *In vitro* experiments on ethanol extracts of callus of *S. rebaudiana* scavenge and detoxify more DPPH free radicals (87.7%), proving its antioxidant potentiality [47]. Anti-oxidative potential of glycoside from three species, *S. Pilosa, S. rebaudiana* and *S. eupatoria* reveals that S. pilosa had more anti-oxidation capacity followed by S. rebaudiana in decreasing the peroxide fat and thus used in the sugar formulation of biscuits with a pleasant taste [48]. Another study based on calmness ratings after and before drinking a tea sample suggest that the tea with nutritive sweetener has a calming effect on consumers compared to non-nutritive sweetener (sucralose or stevia), which may be due to the caloric nature of the sweetener [49]. Noncaloric sweeteners are mainly used for weight loss and glucose intolerance and diabetes [50].

The consumption of natural, non-caloric sweet organic molecule Steviol glycosides (SGs) containing rebaudioside A and aglycon steviol have the potentiality to activate Ca2+-activated cation channel and perception of sweet, bitter and umami taste and enhance glucose-induced insulin secretion TRPM5 which are expressed in type II taste receptor cells and pancreatic β-cells. Their daily intake ceases the development of diabetic hyperglycemia caused mainly due to high-fat-diet in wild helping in controlling type 2 diabetes in Trpm5−/ activated mice [51]. Some studies suggest that stevia extracts are very effective against type 2 diabetic animal models showing insulinotropic and anti-hyperglycaemic effect [52].

Stevia leaf extract containing antihyperglycemic agents stevioside is world widely accepted as supplementary food products for diabetic patients as it regulates blood glucose levels and glucagon secretion, enhances production and impact of insulin on cell membranes, develop potential in glucose tolerance after carbohydrates intake and slows down post-prandial blood sugar levels thus having potentiality to counter type II diabetes in both animals and humans [53-55]. Various proclamation has been put forward on the antidiabetic working mechanism of stevia. A study on wistar rat claims that the plant contain a large fraction of phenol that reduces the Malondialdehyde concentration in liver improving its antioxidant, insulin sensitivity and glucose tolerance capacity; and also improves glomerular filtration rate preventing kidney damage and removing oxidative stress[56].

Stevia leaves in combination with fats and carbohydrates shows synergistic action in decreasing blood-glucose levels and glycemia in rats, another evidence of its antihyperglycemic nature [57]. Stevioside administered orally do not effect body weight, instead improves glucose and insulin levels in rats and causes reduction in expression levels of cytokines, including KC,IL6, IL10, MIP-1α, TNF-α, IL1β, CD11b and CD14, inhibiting the nuclear factor-kappa b signaling pathway in adipose tissue[58]. The stevioside present in the Stevia leaves possess anti-carcinogenesis property as evinced from studies conducted on mouse skin by inducing carcinogens 12-O-tetradecanoylphorbol-13-acetate (TPA), 7,12-dimethylbenz[a]anthracene (DMBA)

and peroxynitrite, which were totally inhibited by this natural agent [59].

CONCLUSION:

In conclusion, stevia leaves as an important source of natural sweetner can be a best substitute for sugar related disoders in humans. The plant should be explored and require more detailed study in humans to clarify its role in energy and weight reduction. The sugar content are maximum in foliage specially in leaves therefore new scientific techniques and tools must be employed to increase content and quality of the chemical composition for better consumption along with its proper use and conservation.

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