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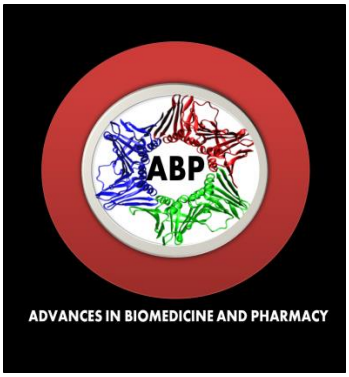

Review Article

Biotechnological approaches in *Stevia rebaudiana* and its Therapeutic Applications

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Abstract

Stevia rebaudiana (Bertoni) is a high value and economic medicinal plant because of its pharmaceutically active compounds throughout the world. Stevia acts as a non-caloric natural-source with therapeutic applications including anti-cariogenic, antimicrobial, anticancer, antioxidant and scavenge free radical and antidiabetic properties. Biotechnological techniques offer novel approaches to the industrial production, propagation, conservation and manipulation of Stevia. The aim of this paper is to describe *Stevia rebaudiana* (Bertoni) biotechnological approaches and its application in foods and medicine.

Keywords: Biotechnology, food, medicine, *Stevia rebaudiana*, Therapeutic.

Introduction

Stevia rebaudiana, is a perennial stemless herb from the Asteraceae family, that is native to South America., it is commercially well known as a high value medicinal plant [1]. Stevia's active components with low calories sweeteners have been shown several useful pharmacological effects such as antioxidative, antihyperglycaemic, anti-hypertensive, antitumor, antidiabetic, anti-HIV which leaves extracts of Stevia [2]. A hectare can produce between 60–70 kg stevioside, which is 300 times sweeter than saccharose and known as honey leaf of sweet chrysanthemum or ''sweet herb of Paraguay'' [3]. Among of about 150-200 species of genus stevia, *Stevia rebaudiana* (Bertoni) with several potential sweetening compounds is very sweet [4]. Many different compounds identified in *Stevia rebaudiana*, which the most abundant are the steviol glycosides, stevioside and rebaudioside A [5]. The stevioside content in leaves controlled by the environmental factors particularly temperature and agronomic management. Das et al., (2010) reported that dry Stevia leaves -were generate more income in tissue cultured propagate established fields than cutting propagated field [6]. Kennelly (2002) reported that the cultivar and growing conditions as two important factors may vary the content of sweet components of Stevia [7]. The concentrations of at least eight steviol glycosides (tetracyclic diterpenes derived

from biosynthetic pathways with gibberellic acid formation) are influenced by the growing conditions and genotype [8] which rebaudioside-A is not much influenced by production environment and agronomic management. Pal et al (2015) reported the best nutritional cause to increase the dry leaf yield [9]. Stevioside and rebaudioside-A are negatively correlated, while rebaudioside-A and -C are positively correlated that the primary aim of plant breeders produces of *S. rebaudiana* with a higher content of rebaudioside-A and a reduced content of stevioside [10]. Quality of Stevia leaves which contained stevioside as natural sweetener extracted is significantly affected by growing conditions such as seasonal variation, radiation, day length, temperature, soil moisture, and harvest time [7]. Tavarini and Angelini (2013) claimed that enhancement of stevioside concentration related to different phases of shoot development, in the field caparisoned stevioside contents among the regenerated plants and the mother plants, founded that leaf stevioside contents were similar between the regenerated plants (10.68 mg/g dry weight) and the mother plants (12.01 mg/g^{-} dw) [11]. The major factors determining in the Stevia production and quality are founded the harvest time specially affecting on the rebaudioside A/stevioside ratio [12].

The leaf of Stevia is a good source of carbohydrates, protein, crude fiber, folic acid, vitamin C and all of the indispensable amino acids with the exception of tryptophan, high percentage of anti-nutritional factors which inhibit the growth of certain bacteria and other infectious organisms [13]. Stevia leaf with potential therapeutic application, nutritional composition and a good source of carbohydrates, protein and crude fiber is natural and safe.

The present review provides a summary on the medicinal properties and biotechnological approaches towards Stevia for understanding its importance in the improvement of this valuable medicinal species in the world.

Tissue Culture

The propagation problem of Stevia is black seeds with higher viability but very low germination potential (heterogeneous plant) thus production of Stevia in large and economic scale requires a huge number of individuals for vegetable propagation via *in vitro* propagation for this problem [14]. More factors involved in tissue culture system for mass production such as genetic make-up of the plant, chemical characters, gelling agent, and also some physical growth factors [15]. Different explants including leaf, shoot tip, nodal segment, inter-nodal segments, apical meristem, flower, axillary bud and roots, also combinations of different plant growth regulators have been employed for plant regeneration in Stevia. Das et al (2011) studied the comparison of three different explants for high frequency multiple shoot induction: shoot tip, nodal segment and axillary bud explants on MS [16] medium basal supplemented with sucrose (30 g/l), agar (7 g/l) and Kn (kinetin) (2 mg/l) that shoot tip induction (85.14 % maximum response) was recorded within 6 days of culture (first bud induction) [17]. Multiple shoot regeneration from the explants of shoot tips and nodal segments followed by rooting of multiplied shoots in two different strengths (full MS and half MS) were studied by Hossain et al (2008) that shoot tips showed better response for shoot proliferation than nodal segments and both of media on shoot proliferation were equally effective [18]. In another study Anbazhagan et al (2010) reported that shoot tip was the best explant in inducing shoot development [19]. Yadav et al (2011) were founded inter-nodal segments initiated callus formation earlier than node and leaf explants [20]. Leaf explants showed better callus initiation than nodal explants [21]. Different explants including leaf, nodal and inter-nodal segments explants on different concentrations of 2,4-D (2, 3, 4 and 5 mg/l) in MS medium assayed by Uddin et al (2006) that callus induction was observed from MS medium with 3.0 mg/l 2,4-D (2,4 Dichlorophenoxy acetic acid) [22]. Different plant growth regulators (PGRs) in several of researches reported such as: Debnath (2008) founded BAP (N6-benzylaminopurine) as a monopolized PGR for multiplication of Stevia (4). Das et al (2011) exhibited a stronger effect of Kn than BAP in shoot multiplication *in vitro*. Hwang, (2006) investigated four basal media: MS, B5 [23], WPM [24], and SH [25] media supplemented with various combinations of auxins and cytokinins have been used but the best performance (23.4 ± 2.1 shoots per explant) was obtained on MS medium containing 2 mg/l IAA (Indole-3-acetic acid) and 0.5 mg/l Kn [26]. Earlier study reported that PGR free MS media was very efficient for *in vitro* rooting of Stevia in comparison of MS supplemented with auxin [17]. A higher concentration of BA (N6-benzyladenine) (2.0 mg/l) reported by Ahmad et al (2010) exerted significant callus induction in comparison of other PGRs and reported that BA induced 70% organogenic response from shoot apex, nodal and leaf explants [27], and maximum number (21.6) of shoots perexplant was recorded for 2.0 mg/l BA, which increase of IAA or 2,4-D in medium content of BA considerably inhibited number of shoots/explant. Consider to this finding Utilization of BA in medium is very affective on mean shoot length, induce organogenic response from different explants and highest number of shoots/explant. Sadeak et al (2009) assayed different auxins including 2,4-D, NAA (Napthalene acetic acid) and IAA at various concentrations in MS media

that showed NAA 0.5 mg/l concentration synchronic shoot proliferation and root induction from nodes explants [28]. 2, 4-D with different concentrations reported lower callus induction on MS medium [29]. Dey et al (2013) was detected application of chlorocholine chloride (CCC) in micropropagation of *S. rebaudiana* Bertoni has commercial prospects [30]. The shoots were successfully rooted with addition auxin at concentration of 0.4 mg/l IBA, IAA or NAA [31]. Previously various studies reported that among of all cytokinins, TDZ (Thidiazuron) (a non-purine cytokinin compound) promoted shoot formation better as compared to others [32]. Various plant-growth regulators could be affected on concentration and compounds of stevioside, steviol and isosteviol or of their metabolites. Flower explants of *S. rebaudiana* used in study of Ahmad et al (2011) were evaluated the effects of various PGRs such as BA in combination with 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) or 0.5 mg/l IAA or 0.5 mg/l GA3 on callogenic and shoot organogenic potential, finally reported best callus induction on MS medium supplemented with 2.0 mg/l BA (93.6%) along with 2.0 mg/l 2,4-D and 0.5 mg/l BA with 2.0 mg/l 2,4-D, 0.5 mg/l GA3 and 0.5 mg/l IAA (85.0%). shoot organogenesis 85.2% recorded from callogenic explants were placed into MS-medium supplemented with 2.0 mg/l BA after 30-days. Selected appropriate PGRs could improve commercial purposes for enhancement and produce of stevioside [29]. Janarthanam et al (2009) founded Significant numbers of shootlets were produced in MS supplemented with 4.44 µM BA and 1.34 µM NAA from juvenile leaf explants of *Stevia rebaudiana* Bertoni [33]. Success in growth and multiple shoot formation reported in present BA media [34]. Fatima and Khan (2010) improved the micropropagation method for *Stevia rubadiana* by incorporation of 1.5 mg/l BA + 0.5 mg/l Kn concentration for shoot induction from apical meristem and nodal explants [35]. Microshoots used as explant in investigated by Dey et al (2013) that MS medium containing different combinations chlorocholine chloride (CCC) and IBA compared to MS medium containing either IBA or CCC, MS medium supplemented with 3 mg/l CCC and 3 mg/l IBA reported most effective [30]. The direct shoot organogenesis with TDZ-Induced efficient on explants in *in vitro* for *Sevia rebaudiana* Bert. Lata et al (2013) expressed maximum multiple shoots (96%) were obtained in MS medium supplemented with 1.0 µM TDZ, which root induction on half strength MS medium without any growth regulator was observed better than others [32]. Janarthanam et al (2009) was observed the highest callus production in MS media containing 0.5 mg/l 2, 4-D, 0.5 mg/l NAA and 1.0 mg/l Kn [33]. In summary, some examples of hormones tested for various explants of Stevia listed in Table 1.

Acclimatization

The plantlets were successfully acclimatized in planting medium containing sand, vermicompost and soil (1:1:1) [36] treated with 0.1% Agrason (fungicide) during 15 days and then were transferred to environmental conditions. Finally, percent of survival 77% reported [37]. Plantlet generated from *in vitro* propagation remarkable acclimatized in a balance mixture of sand, soil and farm yard manure (preservation of moisture) (1: 1: 1 v/v) [17]. Rooted plantlets were hardened on the mixture of cocopeat and moss successful during twenty- five days [38]. Janarthanam et al (2009) for hardening, rooted plantlets were transferred to red soil, vermiculite, and farmyard manure proportional (1: 1: 1) for three weeks. Plantlets acclimatized to small polythene bags containing garden soil and sand (1:1) for eight weeks with 70% growing successfully [33]. Stem node segment explants obtained from 2 years-old shrubs on MS media founded the highest survival percentage (90%) as well as growth percentage to survival (100%) was obtained on MS medium supplemented with 0.5 mg/l BA + 0.5 mg/l (Kin) [39] which conclusively reported 75% of the plantlets survived after kept plastic pots containing sand and peat moss mixture (1: 1) during 2 months.

'Omics' approaches

Biotechnological approaches have opened new windows on the subject of engineering of Stevia sweetness compounds pathway. Genetic diversity assessments can be evaluated variation in subjects such as agronomic, morphological, molecular, biochemical, physiological and other characteristics. Studies on DNA-based molecular markers are in progress in several researches to understanding of the level and partitioning of genetic variation. Different types of molecular markers are proposed for genetic improvement with a wide range of molecular marker technologies. Numerous methods for the evaluation of the genetic variability of genotypes have been employed for the production of stevioside and rebaudioside A of *S. rebaudiana*. Random amplified polymorphic DNA (RAPD), Inter simple sequence repeat (ISSR), Amplified fragment length polymorphism (AFLP) and Simple sequence repeat (SSR) or microsatellites; these methods as widely applicable, fast, inexpensive, require small amounts of template DNA, and high information content. Several studies have been carried out in *Stevia rebaudiana* (Bertoni) to understand the diversity using ISSR, randomly amplified

polymorphic DNA (RAPD) markers Amplified fragment length polymorphism (AFLP) and Simple sequence repeat (SSR).

Influence of genetic variation on morphological parameters among 10 Stevia accessions were studied by Osman and Abdul Lateef (2011) in International Islamic University Malaysia [40]. RAPD-PCR and HPLC methods for the genomic DNA polymorphism and phytochemical variation of *Stevia rebaudiana* Bertoni were investigated; the results suggested that there was a strong correlation between the phytochemical variables and the DNA polymorphism data. Furthermore, stevioside is a major metabolite present in leaves, whereas this sweetening agent concentration linearly correlated to the genetic diversity [41]**.**

Chester et al (2013) studied genetic diversity in 11 accessions of *Stevia rebaudiana* of different geographical regions in India using random amplified polymorphic DNA (RAPD technique) markers and high-performance thin layer chromatography (HPTLC) analysis that they detected 67.24–92.40 percent polymorphism. The information obtained from HPTLC analysis of all samples were carried out on silica using acetone: ethyl acetate: water (5:4:1, v/v/v) showed large variation among samples and the same result obtained with RAPD marker [42]. Assessment of genetic fidelity of *in vitro* propagated *Stevia rebaudiana* plants Based on RAPD analysis were evaluated that no polymorphism was detected with RAPD analysis using DNA from *in vitro*-raised plantlets [41].

In a study of Biotechnological within three samples of Stevia such as RAPD, ISSR (used to evaluate the genetic fidelity of *in vitro* propagated. Lata et al (2013) reported that AFLP was with an average of 30.24% polymorphism between the three samples obtained by all used biochemical and molecular markers [32].

The proportions of rebaudioside-A and -C are controlled by a single additive gene. Complementary genetic studies rebaudioside A and C content clearly revealed that these are synthesized by the same enzyme [10]. Detailed analysis of Stevioside and rebaudioside-A relationship revealed negatively correlated between those, while rebaudioside-A and -C content showed positively correlated, whereas, the ratio of rebaudioside-A to stevioside is the accepted measure of sweetness quality [10].

Steviol glycosides so far have been identified more than 30 additional steviol glycosides are diterpenoids or crop of terpene cyclases (the MEP pathway) [43], the primary source of isopentenyldiphosphate (IPP), 1-deoxy-dxylulose- 5-phosphate synthase (DXS) and 1-deoxy-dxylulose- 5-phosphate reductoisomerase (DXR) are two enzymes catalyzing in pathway. the results obtained in this study showed that the Steviol glycosides were characterized by a high levels of expression of both Copalyl diphosphate synthase (CPS) and kaurene synthase (KS) enzymes are involved in the synthesis of gibberellic acid (GA) and only in leave tissues [44]. Humphrey et al (2006) have been identified four enzymes involved in biosynthesis of steviol glycoside: kaurene oxidase (KO) has a dual role in both steviol and gibberellin biosynthesis and three of the UDP-glucosyltransferases (UGTs) [45]. Studies on the production of Stevia using *in vivo* and *in vitro* cultures reported the production of steviol glycosides in *Stevia rebaudiana* influence by the development of the membrane system of chloroplast which the medium that development membrane system of chloroplast and chlorophyll content require to suitable copper level in MS media [46].

The results indicated efficient transformation system using *bar* gene as a selectable marker for bialaphos herbicide and then produced transgenic resistance of Stevia plants [47]. High yielding transformation of Stevia var. *Spanti* reported 1100 psi (8.3 %) the number of regenerated plantlets/ number of bombarded callus [48]. Pandey et al (2016) indicated *UGT85C2* gene would be a great potential of positive expression in hairy root cultures in regulating the biosynthetic pathway of the stevioside producing [49]. The results of the study *Agrobacterium rhizogenes*: ATCC 15384 and LBA 9402 in *S. rebaudiana* explants showed the type of explants, time of inoculation and concentration play key role in hairy roots formation. Inoculation with LBA 9402 strain of Stevia demonstrated higher efficiency of transformation. In addition, the highest growth of hairy roots was observed in dark conditions [50]. Mandal et al (2013) enhanced the production of the two steviol glycosides: stevioside and rebaudioside-A in *Stevia rebaudiana* via arbuscular mycorrhizal fungi (AMF) through higher concentrations of steviol glycosides. Also they assayed *Rhizophagus fasciculatus* inoculation in *Stevia rebaudiana*, results showed considerable 87% colonies Stevia roots [51].

RNA-Seq, the next generation sequencing technology can be successfully used for gene identification and transcript profiling in a non-model species like Stevia. A comprehensive landscape of the transcriptome profiles of three genotypes of Stevia with divergent steviol glycosides compositions characterized using RNA-seq [52]. Kim et al (2015) integrated metabolome and transcriptome analyses of Stevia to explore the biosynthetic capacity of leaf tissues for diterpenoid metabolism. Tissue-specific chemical analyses confirmed that steviol glycosides were accumulated in leaf cells but not in trichomes [53]. RNA-seq analyses provided a comprehensive overview of dynamic metabolic activities in trichomes and leaf without trichomes. high-throughput small RNAs sequencing to

discover novel microRNAs (miRNAs) has been done by Mandhan and Singh (2015), From 2,509,190 reads, twelve novel miRNAs were predicted whose precursors were potentially generated from Stevia EST and nucleotide sequences [54]. This finding is important to learn more about the roles of miRNAs in Stevia development and physiology but also to provide a framework for further designing RNAi based experiments for regulation of gene expression in these species.

HPTLC as a sensitive, safe, simple and high throughput method developed for quantification of stevioside and rebaudioside A, was used for the quality control of *S. rebaudiana* as well as to check the content of Stevia glycoside during different stages of crop for commercial production [55]. There is inulin-type fructo oligosaccharides as major and significant component of Stevia leave extracts that structure and degree of polymerisation of fructo oligosaccharides present in roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni leaves evaluated by de Oliveira et al (2011) showed high yield (0.46%) of fructooligosaccharides contained (2-1)-linked β-fructofuranosyl, with terminal α-glucopyranosyl and β-fructofuranosyl units [56]. The absolute and relative configurations of novel iminosugar (1R,2S,3R,5R,8aR)-3-(hydroxymethyl)-5-methyloctahydroindolizine-1,2-diol (Steviamine) were examined by Michalik et al (2010) using by X-ray crystallographic analysis [57]. In the ESI mass spectrum, Rebaudioside F showed the molecular formula of $C_{43}H_{68}O_{22}$ (58), also in this research the ^{13}C NMR spectrum indicated signals of a steviol glycoside. Starratt et al (2002) rebaudioside F (steviol derivative) is a trisaccharide residue consisting of two glucoses and a xylose unit linked through oxygen to C-13 [58]. On the basis of NMR and MS studies Prakash Chaturvedula and Prakash (2011) identified 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] *ent*-kaur-16-en-19-oic acid-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl) ester as a new diterpene glycoside compound from *Stevia rebaudiuana* [59]. Chlorogenic acids, aglycons and flavonoid glycosides of *S. rebaudiana* from seven different botanical varieties were profiled and quantified in a total of 166 samples. Principal component analyses allow distinction between varieties of different geographical origin and distinction between different plant varieties [60].

Medicinal properties of Stevia

The leaves of Stevia have different compounds with many medical applications including stevioside (St), rebaudioside (Rb) A, B, C, D and E, dulcoside A and steviol biosides that their compounds without side effects improving the nutrient properties [61]. *In vitro* and *in vivo* grown Stevia leaf extracts in different solvent system were screened for potential antimicrobial activity against medically important bacterial and fungal strains. A concentration dependent antibacterial and antifungal inhibition have been shown which can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation [4]. Many countries use Stevia as ethnomedicinal usage such as Brazil (diabetes, fatigue, heart support, hypertension, hyperglycemia, infections, obesity and sweet cravings), United States (diabetes, hypertension, hyperglycemia, infections), Paraguay (diabetes) and South America (diabetes, hypertension, infections, obesity).

Being a non-carbohydrate sweetener, Stevia would not favor the growth of *Streptococcus mutans* bacteria in the mouth which is attributed to be a causative agent of dental caries and tooth cavities. Certain compounds in Stevia inhibit caries causing bacteria in the mouth [62]. *In vitro* researches have shown that the acetone extracts show higher antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholera* [63]. It has been found that inhibitory activity *in vitro* of extracts from Stevia leaves with 200 mg/ml concentration on *Streptococcus mutans* [64]. Ghosh et al (2008) assayed antimicrobial properties of Stevia plant extracts against pathogenic species using six solvents against ten selected pathogenic including four type of fungal and six type of bacterial, highest antifungal index AfI-15mm and antibacterial index AbI-11.2mm were obtained for petroleum ether extract against all pathogens. Besides, the cyclo-hexane, acetone and ethanol did not show anti-fungal effects [65].

Antioxidant

Several studies revealed that the medicinal benefits of Stevia leaf extracts (promote health) with remarkable antioxidant compounds via removing properties of the free radicals and reactive oxygen species (ROS) [66]. They represented the total phenolic and flavonoid contents are two significant agents of the antioxidant potential. The major agents contributing towards the antihyperglycemic activities reported that phenols exist in Stevia extract. The total flavonoid content analyzed by Kennelly et al (2002) detected the flavonols and flavones in Stevia leaves [67]. Another compounds of scavenging free radicals in Stevia including ascorbic acid, glutathione, uric acid, tocopherols, carotenoids, polyphenols, and non-sweetest compositions. Based on data obtained from Lopez et al (2016) antioxidant activity of Stevia was not due to stevioside present also their results shown antiproliferative activity of Stevia [68].

Vasko et al (2014) expressed exhibiting and scavenging capacity with increasing concentrations of extract [69]. The dried leaves of Stevia from five different geographical locations of India were estimated with the help of DPPH free radical scavenging assay, its result introduced the best variety with the highest phenolic content 5.87mg GAE/L and flavonoid content 62.22 mg GAE/L from Kangra. Šic Žlabur et al (2015) claimed that the positively link relationship between antioxidant activity and the total amount of phenolic content. The optimum conditions for the ultrasound treatment on *Stevia rebaudiana* Bertoni leaves extraction for the steviol glycosides, total phenolic compounds, and flavonoids Šic Žlabur et al (2015) expressed: extraction time 10 min, probe diameter 22 mm, and temperature 81.2oC [70]. Commercial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and Tertiary butyl hydroquinone (TBHQ) caparisoned with antioxidant property of Stevia leaves that the conclude showed Stevia leave had higher antioxidant potential. An increase in the level of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) observed with use of Stevia, specially SOD and CAT as primary antioxidant enzymes [71]. The biosynthesis of GSH increased with the leaf extract which thereby reduces the oxidative stress. They can scavenge radicals such as singlet hydroxyl, superoxide and oxygen radicals. Vitamin E (α-tocopherol) impresses in free radical scavenging, xanthine oxidase inhibitory and anti-lipid peroxidation activities.

Anti-diabetic

The extracts of *Stevioside rebaudiana* could decrease the blood glucose level in diabetic rats in time dependent manner; the antidiabetic effect might be due to steviosides counteracting the glucotoxicity in β-cells or also by suppressing the glucagon secretion by α-cell of pancreas [72]. Stevia leaves have a significant role in alleviating liver and kidney damage in the STZ-diabetic rats besides its hypoglycemic effect; it could protect rats against streptozotocin induced diabetes, reduce the risk of oxidative stress and ameliorate liver and kidney damage [71]. Stevioside and rebaudioside A, the main sweetening metabolites in Stevia leaves can act as a source of antioxidants. An important part of the medicinal Stevia is against diabetic patients and destroying the harmful effect of sugar [73]. They carried out on alloxan-induced diabetic rats, Stevia extract at daily dose of 200 and 400 mg/kg body weight and duration 10 days was found significant decrease in the blood glucose level. Additional, they indicated reduction in body weight with medium-polar (benzene:acetone, 1:1, v/v) extract of *S. rebaudiana* leaves, it could be not taken up by the human body. Studies on the protective mechanism at the cell membrane level in diabetic rats observed increase of the vitamin E and C level with the synergic relation as antioxidant properties [71]. Shivanna et al (2013) confirmed this positive effect on diabetic patient [71]. Stevia could prove to be beneficial in hypertensive patients by inhibition of Ca^{2+} channel from the extracellular fluid into the blood vessels [74]. In animal study, the role of methanolic leaf extract of *S. rebaudiana* for 21 days in managing the diabetic complications in alloxan induced mice, their results revealed clearly reduction in the sugar level, the level of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein. It could be stimulating the beta cells of langerhans to release insulin thus as well as the carbohydrate metabolizing enzymes start. They also showed increase in the level of GPx suggesting its antioxidant effects. [75]. Stevioside transport is very low but steviol by passive diffusion and carrier-mediated transport is much faster. Stevioside inhibiting glucagon secretion from α cell of pancreas with effects on both gluconeogenesis and glycogenolysis, afterward direct suppression of phosphoenol pyruvate carboxy kinase (PEPCK) activity. stevioside, steviol and rebaudioside an increase insulin secretion from β-cells of the pancreas and enhance glucose uptake afterward they exhibit anti-hyperglycemic action by reducing glucose production while increasing glucose uptake by the intestine into the blood [76].

Anti-cancer

According to Mizushina et al (2005) report, isosteviol from stevioside inhibited DNA replication and human cancer cell with inhibition of two significant enzymes: mammalian DNA polymerases and human DNA topoisomerase II thus isosteviol strongly provide cancer therapy [77]. Deshmukh and Kedari (2014) founded the methanol and ethanol extracts of stevioside can be used to anticancer activity against Caco-2andCaski cell lines as compared to water extracts [78]. Yuajit et al (2013) revealed steviol inhibiting Madin-Darby canine kidney (MDCK) cyst growth by reducing cystic fibrosis trans membrane conductance regulator (CFTR) expression levels that this inhibition related to used dose of Stevia and expressed dose up to 200 microM had no effect on MDCK cell. stevioside, rebaudiosides A and C and ducloside A of *Stevia rebaudiana* have the growth inhibitory activity or anticancer effects. The antitumor activity has been observed in studies conducted in Human laryngeal epithiloma cells (HEp2), the MTT assay (measured the cell viability) for treated cells by

acetone extract of *Stevia rebaudiana* showed that 1:8 dilution of the acetone extract (the effective drug concentration) had anticancer and anti-proliferative activities against abnormal cells [79]. Some researchers suggested that polyphenol, antioxidant and anti-inflammatory effects of Stevia. Evidential research reports indicate that Stevioside inhibits TPA-induced tumor promotion in a skin cancer model in mice [80].

Renal protective

Stevioside and steviol induce diuresis and natriuresis, without a significant change in glomerular filtration rate or renal plasma flow [81]. It was found that in the diabetic rats fed with Stevia on renal function observed remarkable reduction in the glomerular filtration rate (GFR) and renal plasma flow (RPF). Melis et al (2009) investigated Steviol (the aglycone compound of Stevia) excretion from the renal tubule, steviol was infused at three doses 0.5, 1.0 and 3.0 mg/kg/h in rats that they observed steviol causes increase in the fractional sodium excretion, fractional potassium excretion, urinary flow as percent of glomerular filtration rate (V/GFR) and glucose clearance [81]. The effect of steviol on renal cyst growth in an orthologous mouse model of autosomal dominant polycystic kidney disease (ADPKD) studied by Yuajit et al (2014) steviol and stevioside decreased kidney weight and cystic index in these mice and steviol slows cyst progression in ADPKD mouse model and improved renal function [82].

Conclusions

In conclusion, *Stevia rebaudiana* (Bertoni) as a well-known medicinal plant has been used for various purposes. Stevia has a wide range of compounds including steviosides and their related compounds such as rebaudioside A-E, steviolbioside and isosteviol which could be responsible for the plant's sweet taste and therapeutic benefits.

Table 1: Effect of Hormonal treatment (mg/l) for plant regeneration from different explants of Stevia.

Hormonal treatment (mg/l)	Explant	Response	Media	Reference
Kn (2 mg/l)	shoot tip	85.14 % increase within 6 days of culture	MS	[17]
2,4-D (3.0 mg/l)	leaf, nodal and inter-nodal segments	callus induction	MS	[22]
BA	shoot apex, nodal and leaf	70% organogenic response	MS	[27]
IBA (0.4 mg/l) + IAA or NAA	shoots	rooted	MS	[31]
NAA (0.5 mg/l)	nodes	synchronic shoot proliferation and root induction	MS	[28]
BA (2.0 mg/l) + 2,4-D (2.0 mg/l) and BA (0.5 mg/l) + 2,4-D (2.0 mg/l) and GA3 (0.5 mg/l) + IAA (0.5 mg/l)	flower	callus induction	MS	[29]
BA (2.0 mg/l)	callogenic explants	shoot organogenesis 85.2%	MS	[29]
IAA (1.0 mg/l)	shoots	root formation	Half-strength Nitsch (N6)	[19]
BA (4.44 µM) + NAA (1.34 µM)	juvenile leaf	shootlets	MS	[33]

BA (1.5 mg/l) + Kn (0.5 mg/l)	apical meristem and nodal	shoot induction	MS	[35]
IAA (2 mg/l) + Kn (0.5 mg/l)	shoots		MS	[11]
3 mg/l CCC and 3 mg/l IBA	Microshoots		MS	[30]

Table 2: Medicinal properties of Stevia compounds.

Compound	Medicinal properties	Reference
Stevioside	Anti-hyperglycemic effect	[80]
	Anti-inflammatory effect	[83]
	Hypotensive effect	[84]
Rebaudioside (Rb) A	Positive effects on PTZ-induced convulsions	[85]
Dulcoside A	Glycemic Effects	[86]
Steviol	Renal function	[81]

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