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*Review Article*

# *Stevia rebaudiana*, a Versatile Food Ingredient: The Chemical Composition and Medicinal Properties

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*Stevia rebaudiana* is a well-known sugar substitute with low calories. Recently, Stevia rebaudiana has been reported with many medicinal properties that could possibly be used in functional foods. The work presents the chemical composition of Stevia rebaudiana. The Stevia is capable of treating renal diseases, liver pathologies, diabetes, hyperglycemia, hypoglycemia, hypertension, tumors, microbial diseases, and obesity. Nonetheless, researches are still going on to understand Stevia. Therefore, the conclusion made is not everything known about Stevia. This work highlights chemical composition of Stevia rebaudiana and its medicinal properties like antioxidant, antidiabetic, anti-inflammatory, antihypoglycaemic, anticancer, renal-healing, cardiac-healing, antilipidemic, and hepatoprotective effects. The information has been discussed from a food and medical perspective. Future works may focus on the development of novel functional foods.

## 1. Introduction

Nature is full of unveiled scientific inventions, innovations, and discoveries. There are about 1500 years now since *S. rebaudiana* (a food ingredient) had been used by our antique forefathers. Dr. Moises Santiago Bertoni, who was a Director of Agriculture in Asuncion College, discovered *Stevia* in Paraguay in 1888. He first gave the biological description of *Stevia*. Few years down the line in 1905, Dr. Rebaudi, a chemist named the plant (Momtazi et al., 2016). He was the first person to extract and isolate the glycosides in the *Stevia*. Complete characterization of the glycosides was done by 1931. *Stevia rebaudiana* appertains to a genus of *Asteraceae* family. It is one of the 154 known members of this family. It belongs to Kingdom *Plantae*, *Angiosperms* division, *Eudicots* class *Asterals* order, *Stevia* genus, *Eupatoricae* tribe, and *Bertholdii* species (Verma et al., 2019). This green herb has elliptical, sessile leaves which are approximately 3-4 cm long. It can grow up to 1 m in terms of height (Fasiha et al., 2020). Around 1961, cultivation of the herb commenced [1]. The herb is native in Amambay Valley, a junction formed by some regions of Argentina, Brazil, and Paraguay. In Brazil and Paraguay, *Stevia* is found naturally between 22°-24° south and 53°-54° west. Paraguayan Indians are the pioneers in using *S. rebaudiana* leaves as natural sweeteners. *S. rebaudiana* leaves are referred to as candy or honey leaf because of its sweet taste. It is reported that only a handful leaves are enough to increase the sweetness of beverages, for example herbal tea. The use of the leaves became common and popular since the discovery made by the Paraguayan Indians that the *S. rebaudiana* leaves can be used as natural sweeteners. Long back, the herb seemed only to be more medicinal than commercial. *S. rebaudiana* is now known as an important commercial herb. Almost every continent is actually aware of this practice and started *Stevia* agriculture, following the observation that it is a potent medicinal and commercial herb (Zou et al., 2020). These nations include China, Canada, Indonesia, Korea, Brazil, Mexico, Tanzania, and United States of America (Martins et al., 2016; Zou et al., 2020). Approximately 1000000

hectares worldwide of Stevia cultivation were reported, with China having the largest portion (Sharangi et al., 2016).

Among about 200 genus species of Stevia, *S. rebaudiana* has drawn a great portion of attention due to its sweet nature (Ahmed & Mukta, 2017). *Stevia* flourishes in an acidic-neutral (roughly pH 6-7) soil rich in animal or plant remains. Moisture is needed. Researches revealed that *Stevia* grows naturally in low lying sand acidic parts next to swamps. Application of urea fertilizer ought to be done thrice. First application is done at basal. The rest two are done following first and second cutting of leaves. Phosphorus and potassium high levels plus low nitrogen level enhance Stevia flourishment. Borax 6% is sprayed whenever the plant shows boron deficiency ailment symptoms. The disease is known for causing spots on the leaves [1]. Researches are still going on to understand the herb fully and maximize on it. There are several factors that affect the amount of stevia secondary metabolites, including the nutrition and climate. For example, high glycoside percentage is found in Stevia grown in mountainous regions. It is reported that when length of the day is less than twelve hours and when temperatures are below 293 K, the rate of growth diminishes. Increasing day length to approximately sixteen hours and elevating light intensity increases growth and stevioside levels. Nonetheless, a number of varieties have a disposition not to give positive phototropism response. Stevioside content is higher in early flowering stevia but the total yield is lower. Researchers unveiled the observation that stevioside concentration in the leaves increase when the plant is grown under long day conditions. Also, transplanting and planting dates are critical agricultural factors that have a big likelihood to quantity and quality of yield [1]. The purpose of this review is to delve into the distribution, chemical composition, medicinal uses, pharmacology, and commercial aspects of Stevia.

## 2. Chemical Composition

Momtazi et al. (2016) assert that in some studies and experiments conducted, *Stevia* leaves are loaded with different types of chemicals. Among the chemicals are nine essential amino acids viz. glutamate, aspartate, methionine, tyrosine, proline, alanine, isoleucine, lysine, and serine. Lemus-Mondaca et al. (2012) reported that all essential amino acids are present in the leaves of Stevia with an exception of tryptophan. In addition to that, fatty acids, namely, linoleic acid, linolenic, oleic, stearic, palmitoleic, and palmitic acid, were found present. Moreover, vitamins such as vitamin $B_{12}$, vitamin C, and folic acid were also present, along with minerals such as calcium, phosphorus, magnesium, iron, sodium, zinc, and potassium. Not only that, phytochemicals, namely, $\beta$-carotene, thiamine, steviol, stevioside, riboflavin, rebaudi oxides, nilacin, dulcoside, and austroinullin, were detected in the plant leaves. Needless to say, secondary metabolites are present. Majority of articles and researches report that anthraquinones, reducing compounds, triterpenes, sterols, saponins, cardiac glycosides alkaloids, and tannins are found in the *Stevia* leaves. Terpenes and flavonoids dominate in terms of chemical composition in the *Stevia* leaves (Sharangi et al., 2016). Fasiha et al. (2020) reported the presence of phenols and coumarins in the *Stevia* leaves.

Glycosides found in *Stevia* have a common backbone structure, which are given an umbrella term, steviol glycoside. These chemicals are diterpenes with four rings. The sweet taste is rendered by the C-13 hydroxyl group and C-19 carboxyl group. Stevioside, steviolbioside, isosteviol, and rebaudioside A, B, C, D, E, and F are the major glycosides present in the herb. Stevioside and rebaudioside C and A are 250-300, 50-120, and 250-450 times sweeter than can sugar, respectively. Rebaudioside A and D are convertible to rebaudioside B under alkaline hydrolysis. The duo is highly soluble and can be metabolized in the human body without side effects. The most attractive attributes of the two are that they are thermostable (up to 200°C), highly water soluble, pH stable, and do not ferment (Sharangi et al., 2016). On the next leaf is a backbone structure of glycosides found in Stevia as reported by Lemus-Mondaca et al. (2012). Different group constituent results in different glycosides are shown in Table 1.

## 3. Methods Employed for Extraction and Identification of a Broad Spectrum of Chemicals in *Stevia*

A lot of different extraction methods had been employed and can be categorized as solvent extraction (Bondarev et al., 2001; Morita et al., 1978), supercritical fluids (Keinle, 1992), selective precipitation (Fuh & Chiang, 1990), ion exchange, and chromatographic adsorption (Herrera et al., 2001; Nathalie *et al*., 2019). Complex compounds extracted from *Stevia* leaves vary in terms of composition. Concentrations of these complex compounds depend on the herb genetic make-up and the region of cultivation [3]. For instance, Khiraoui et al. (2017) reported that the variations are due to geographical locations and cultivars. Gasmalla et al. (2014) contributed other factors which affect *Stevia* chemical composition. The researchers observed that methods of drying and processing affected percentage chemical yield.

*3.1. Steviosides.* Solvent partition extraction is the refining standard operating procedures for the extraction of stevioside from *Stevia*, which were grouped into water and methanol, by Rank and Midmore (2006). The methods encompassed solvent partition extraction coupled with in situ precipitation with calcium hydroxide-carbon dioxide to get rid of the impurities. The duo seemed to employ the exact purification procedure that is employed in the sugar industry (Lemus-Mondaca et al., 2012). In addition to that, Rank and Midmore (2006) reported other methods of purification like plasmid gel, ion exchange, chromatographic techniques, and adsorption activated by graphite. Hot water was the extraction medium with first preference. The reason being that rebaudiside A was more soluble than stevioside (Liu et al., 1997). Nonetheless, a number of patents highlighted good reasons in using solvents like methanol, ethanol, chloroform, sorbitol, glycerine, and propylene glycol (Lemus-Mondaca et al., 2012). Liu and colleagues (1997) isolated stevioside from *S. rebaudiana* dried leaves using hot methanol. The team went on further to study the

Table 1: The substituents of steviol glycosides.

| Steviol glycoside | R1 constituent | R2 constituent |
|---|---|---|
| Dulcoside A | β-Glc | β-Glc-α- Rha(2 → 1) |
| Rebaudioside A | β-Glc | β-Glc-β-Glc(2→1) β-Glc(3→1) |
| Rebaudioside B | H | β-Glc-β-Glc(2→1) β-Glc(3→1) |
| Rebaudioside C | β-Glc | β-Glc-α-Rha(2→1) β-Glc(3→1) |
| Rebaudioside D | β-Glc-β-Glc(2 → 1) | β-Glc-β-Glc (2→1) β-Glc (3→1) |
| Rebaudioside E | β-Glc-β-Glc(2 → 1) | β-Glc-β-Glc(2 → 1) |
| Rebaudioside F | β-Glc | β-Glc-β-Xyl(2→1) β-Glc(3→1) |
| Stevioside | β-Glc | β-Glc-β-Glc (2 → 1) |
| Steviolbioside | H | β-Glc-β-Glc (2 → 1) |
| Steviol | H | H |

Key: Glc: glucose; Rha: rhamnose; Xyl: xylose [2].

extraction of steviol glycosides such as dulcoside A, revaudioside C, and rebaudioside A using subcritical fluid extraction (Sub FE). Liu et al. (1997) devised a simple Sub FE efficient method and at least 88% efficiency was attainable using methanol modifier.

Pol et al. (2007) reported that stevioside is commonly extracted using hot water, leaching, or supercritical fluid extraction (SFE), followed by a quantitative analysis of extracts to examine its purity using chromatographic techniques such as recrystallization coupled with preparative reversed phase-high-performance liquid chromatography (RP-HPLC) (Nathalie et al., 2019). The above method is slower as to the SFE method that utilizes carbon dioxide as a medium for extraction. The method has supercritical carbon dioxide physicochemical advantages like lower viscosity and higher diffusivity properties as compared to conventional liquid solvents. Notwithstanding, carbon dioxide in its pure form is destitute of suffice solvation strength for stevioside, a polar organic compound. Consequently, addition of a polar cosolvent is imperative. Probed copolar solvents include water, methanol, ethanol, and mixtures of diverse proportions of these solvents (Yoda et al., 2003; Choi *et al*., 2002, Pasquel et al., 2002 and Abau-Arab *et al*., 2010). An alternative approach is the method reported by Jimenez et al. that separates glycosidic compounds, following aqueous extraction, on the basis of molecular weight. The process was done through an amino column using HPLC, where findings were reported in percentage relative standards (Lemus-Mondaca et al., 2012). Afandi et al. (2013) devised a solvent extraction method to isolate stevioside from *S. rebaudiana* leaf powder. The team used solvents such as diethyl-ether, petroleum ether, methanol, and butanol. The purification stage was performed using HPLC to get rebaudioside, a bioactive compound. Interestingly, Rao et al. (2012) carried out the isolation and purification of steviosides from a different angle. They ground the dried leaves, removed all fats, and then carried out the extraction process using pressurized hot water extractor (PHWE). The final stage which required concentration and purification of sweet glycosides was conducted using nano- and ultramembrane filtration. Rao and colleagues managed to achieve 98.2% purity of the steviosides.

*3.2. Phenols.* Chlorogenic acids, which fall under polyphenol esters family, are the major constituents of polyphenols found in *Stevia rebaudiana*. Examples are hydrocinnamic acids and quinic acids (Myint et al., 2020). Pacifico et al. (2019) reported isochlorogenic acids and hyrocinnamic acids as part and parcel of polyphenols present in *Stevia* leaves. Generally, phenolic compounds are vital for plant growth and defend against injuries and infections, rendering oxidative stability (Pacifico et al., 2019; Arriola et al., 2019). Singh et al. (2008) and Hanhineva et al. (2010) defined polyphenols as dietary antioxidants, which include polyphenols from grape seeds tea and apples. Can and Baltas (2016), Lemus-Mondaca et al. (2018), Kim et al. (2011), Zheng et al. (2017), and Pacifico et al. (2019) confirmed the presence of the following polyphenols in *Stevia rebaudiana* (Bertoni) leaves: caffeic acid; 4-coumaric acid; cinnamic acid; syringic acid; vanillic acid; 4-methoxybenzoic acid; 4-methylcatechol; gallic acid; pyrogallol; tricaffeoylquinic acid; 3,4,5-tricaffeoylquinic acid; 1,3,5-tricaffeoylquinic acid; rutin; 3-feruloyl-5-caffeoylquinic acid; 4-caffeoyl-5-feruloylquinic acid; 3.5-dicaffeoylquinic acid (isochlorogenic acid A); 1,4-dicaffeoylquinic acid; 3,4-dicaffeoylquinic acid (isochlorogenic acid B); 1,3-dicaffeoylquinic acid; 4,5-dicaffeoylquinic acid (isochlorogenic acid C); quercetin-3-O-glycoside; galuteolin; quercetin; roseoside; 3-feruloylquinic acid; 5-feruloylquinic acid; 4-caffeoylquinic acid (cryptochlorogenic acid); 3-caffeoylquinic acid (chlorogenic acid); 4-caffeoylshikimic acid; 3-caffeoylshikimic acid; 5-caffeoylquinic acid (neochlorogenic acid); 5-p-coumaroylquinic acid; 5-caffeoylshikimic acid; catechin; luteolin, sinapic acid, and *trans*-ferulic acid (Myint et al., 2020).

The methods employed for the extraction and determination of polyphenols varied from researcher to researcher. On one end of the spectrum, Wolwer-Rich (2012), Kim et al. (2011), and Pacifico et al. (2019) regarded liquid chromatography-tandem mass spectrometry (LC-MS/MS) as the most reliable and effective method for the determination of polyphenols. Oppositely, Myint et al. (2020) observed that LC-MS/MS analysis was not that much reliable when it comes to quantification of phenols. The reason is that the method requires many chemicals as standards since there are many polyphenolic isomers. Consequently, other methods were needed to accomplish the quantification and characterization of phenols. These methods included NMR (nuclear magnetic resonance) and purification processes. Nowadays, Folin-Ciocalteu assay had been widely used for the total phenolic content (TPC) analysis.

The assay works on the basis of calorimetric redox reactions performed by phenols. Folin-Ciocalteu is the frequently used assay owing to its ease of use and cost. The total phenolic content (TPC) may be reported as the equivalent standard chemical, like gallic acid (GA), catechin (CE), and tannic acid

(TA), with the unit of milligrams or micrograms equivalents per gram of dried extract. The extracts can be 'named' catechin equivalent, gallic acid equivalent, etc. (Myint et al., 2020). Notwithstanding, the assay has its drawbacks. One of the major disadvantages is that the assay has an inability to select polyphenols with respect to equivalent standard chemicals. A subtle task to choose the rightful reference chemicals as standards is crucial due to the discrepancies in polyphenolic molecular weights and standard molecular weight equivalent. Myint et al. (2020) illustrated the major disadvantage of the assay by analyzing a sample that was from Zhucheng Hao Tian Pharm Co. Ltd., Shandong, China. The sample was labelled 'no chlorogenic acid' and 58.92% TPC. Now, upon analysis and chemical composition determination by the HPLC method, the sample contained 36.98% isochlorogenic acid A, 16.50% isochlorogenic acid C, 4.50% isochlorogenic acid B, 0.69% chlorogenic acid, 0.15% neochlorogenic acid, and 0.10% cryptochlorogenic acid. The sample could be labelled 771 mg GAE/g TPC. The same sample could be also 1.076 mg chlorogenic acid equivalents per gram extract. The 1.076 mg anomaly is explained by the fact that chlorogenic acid offers much lower molecular weight. In addition to the above reported chemical composition, the sample was found to contain 31.64% polysaccharides which were detected by phenol-sulphuric acid assay, 0.44% total flavonoid, and 0.82% protein, determined by the Kjeldahl method. Another striking example to shed some light on how scientists ought to be careful when conducting the Folin-Ciocalteu assay is the discrepancies in TPC reported by Karakose et al. (2011) and Kim et al. (2011). Karakose and colleagues extracted chlorogenic acids using chloroform-methanol solvent. After that, further determination process was carried out using the LC-MS method. The team reported twenty-four chlorogenic acids which were mainly hydoxycinnamic acid derivatives of shikimic and quinic acid. Altogether, chlorogenic acids amounted to 370 $\mu$g GAE/g of dry extract. Kim et al. (2011) used a water extract and observed that the major phenolic compound in both callus and leaf was pyrogallol, 0.04 mg/g extract in callus, and 9.51 mg/g in leaf extracts. The team reported a sum of 130.76 mg catechin (CE).

As illustrated above, the unwanted substances act as impurities during the quantification and characterization of polyphenols. Substances like sugars, ferrous ion, organic acids, reductones, other enediols, ascorbic acid, sulphur dioxide, and aromatic amines react with the Folin-Ciocalteu reagent giving rise to biased results (Prior, Wu & Schaich, 2005). Consequently, such substances should be removed using a broad spectrum of purification methods for more accurate results.

## 4. Health-Promoting Activity of *Stevia* Phenolic Compounds

According to Zhang et al. [4], *Stevia* leaves are safe to eat. Majority of polyphenols therein have antioxidant activity (Lemus-Mondaca et al., 2018). In addition to that, anticancer, antilipidemic, anti-inflammatory, and antidiabetic properties are reported (Liang & Kitts, 2016; Gouthamchandra et al., 2017; Ong, Hsu & Tan 2013; Pacifico et al., 2019; [3]; Zaidan et al., 2018). Phenolic compounds come in different classes. Among the different classes, phenolic acid derivatives, anthocyanins and flavonoids are the major ones in terms of abundancy. The trio is synthesized in the chloroplasts via a pathway known as the phenylpropanoid synthetic pathway (Santiago et al., 2000; [5]; Khoddami et al., 2013). The chemical compounds are known for protecting the plants from insects, viral, and bacterial invasion (Nicholson & Hammerschmidt, 1992; [6]; Lee et al., 2004; Baidez *et al*., 2007, Eyles *et al*., 2010; [7]). Not only that, the phenolic compounds have the ability to safeguard the plant deoxyribonucleic acid (DNA) by protecting it from oxidative reactions. Mitigation of photooxidative harm in photosystems is executed by the phenolic compounds (Zhang et al., 2018). Many published papers report that increased light intensity correlates with an upregulation of phenolic compounds [4, 8].

*4.1. Antioxidant Activities.* Biological systems have complicated metabolic processes. Reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anion are produced along with the metabolic processes. If a body experiences an imbalance between free radicals produced and the ability of the body to get rid of the radicals, serious health conditions arise due to oxidative stress. For example, cardiovascular pathologies and cancer arise if the ROS persist in the biological systems. The biological systems are loaded with mechanisms meant to prevent any harm that might be caused by ROS. The mechanisms can be categorized into two: enzymatic and nonenzymatic systems. Biological systems have enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase which demolish ROS and other free radicals. Albumin and bilirubin are endogenous nonenzymatic antioxidants which serve the same purpose as that of the aforementioned enzymes. Nonetheless, phenomena where the ROS outpower the endogenous antioxidant systems occur. Exogenous antioxidants are in such times imperative. Sources of exogenous antioxidants include supplements, pharmaceuticals, and food. Among exogenous antioxidants are ascorbic acid, phenolic compounds, carotenoids, and minerals like zinc and selenium (Santos-Sanchez et al. 2019).

*4.1.1. Oxidative Stress.* Oxygen molecule is crucial. It is involved in aerobic cell metabolism to produce energy needed to sustain life. The downside of the molecule is that it can pose serious harm in the body due to its paramagnetic properties. Partly oxidized intermediates, which are highly reactive, are formed due to the paramagnetic properties of the molecule. Such intermediates are referred to as reactive oxygen species (ROS). Exogenous factors like ultraviolet radiation contribute to the formation of ROS. The ultraviolet (UV) light has potency to lyse molecules. In case of the oxygen molecule, homolysis occurs, giving rise to oxygen radicals. ROS can be formed during the course of an ailment. Progression of diseases like myocardial infarction produces free radicals. Biotransformation of xenocompounds carried out in the body by a concert of defense molecules also forms free radicals reluctantly, following chemical intoxication. The purpose of biotransformation is to mitigate the toxicity

of the foreign compound hence rendering it ineffective. Nonetheless, the unexpected happens (Santos-Sanchez et al., 2019).

The immune system carries out inflammatory response upon the invasion by foreign bodies. There is a production of free radicals which destroy foreign bodies. However, detrimental results ensue when the inflammatory response last longer than expected. Immune cells like macrophages and neutrophils make use of the NADH oxidase system to get rid of foreign bodies. The cells, in doing so, produce superoxide ion, a free radical. The superoxide ion is regarded as a primary ROS. The ion can then undergo some reactions to form a secondary ROS. Enzymatic reactions and metal ions are examples of two systems that are capable of reaction with a primary superoxide ion, forming a secondary superoxide ion ROS. Photolysis of water is one the reactions known for the generation of a superoxide ion. When the superoxide ion undergoes reactions like protonation, $H_2O_2$, and $HO_2$·, ROS are produced (Santos-Sanchez et al., 2019).

The action of nitric oxide synthase on intracellular arginine, as a defense mechanism executed by immune system cells, leads to the formation of .NO radical. If the formed radical merges with $O_2$, the ONOO· radical is formed. It is the ONOO· radical that induces lipoprotein peroxidation. Individuals suffering from the following autoimmune diseases have significant lipoprotein oxidation rate once occurring: vitiligo, psoriasis, multiple sclerosis, scleroderma, inflammatory bowel disease, Hashimoto's disease, Grave's disease, celiac disease, type 1 diabetes, primary biliary cirrhosis, systemic lupus erythematosus, and rheumatoid arthritis (Santos-Sanchez et al., 2019).

Sometimes free radical production is good and crucial in some biochemical reactions. Giving examples, production of polypeptides requires free radicals. The free radicals aid in formation and chain elongation of proteins during amino acid polymerization. Also, glycogenesis requires free radicals. A number of enzymes that are responsible for the formation of intermediary molecules require free radicals for activation. Examples of such enzymes are lipoxygenase, cyclooxygenase, monoamine oxidase, aldehyde oxidase, xanthine oxidase, and hypoxanthine (Santos-Sanchez et al., 2019).

Structural changes in critical biomolecules, like lipids, proteins, and deoxyribonucleic acid caused by irreversible reactions, lead to the production of free radicals. Such chemical reactions cause the formation of hydroperoxides and monoaldehyde derivatives that propagate oxidative damage. Apart from ROS, there are reactive nitrogen species (RNS). RNS are generated in small amounts during cellular processes like regulation of cell growth, production of cellular energy, phagocytosis, immune system control, blood pressure modulation, platelet aggregation, peristalsis, muscle relaxation, neurotransmission, and cell signaling. Examples of RNS are NO· (nitrogen oxide), $NO_2$. (Nitrogen dioxide), $ONOO^-$ (peroxynitrite), $ONOOCO_2^-$ (nitrosoperoxycarbonate), $NO_2^+$ (nitronium ions), peroxynitrous acid (ONOOH), and dinitrogen trioxide ($N_2O_3$) (Santos-Sanchez et al., 2019).

Below is Table 2 that sums up the sources and potential harm that might occur to biomolecules in the body.

*4.1.2. Assays and Mechanisms of Antioxidant Action.* There is a very thin line between oxidant capacity and activity. Majority of scientists use the two terms interchangeably yet they do not mean the same thing. When studying mechanisms and antioxidants assays, great care ought to be taken concerning the confused two terms. A rate constant of reaction between an antioxidant and an oxidant is called antioxidant activity. Antioxidant capacity is defined as the measure of how much free radicals an antioxidant sample can capture. When selecting method, the consideration of response parameter is crucial in terms of evaluating the antioxidant properties of a sample. The responses parameter could be a function of the substrate's concentration, or the concentration and time required for the inhibition of ROS with defined concentration. For the environment in which the free radical (FR) is, its structure and reactivity govern the antioxidation reaction mechanism to be employed for such particular FR. It is imperative therefore to describe ROS and the reactive nitrogen species (RNS), to a lesser extent, which include both free radicals and precursors (Santos-Sanchez et al. 2019).

The phenolic-compound measurement of antioxidant potency can be categorized into two: (a) electron transfer ability as like ferric-reducing antioxidant power, radical scavenging of 2,2′-azino-bis-ethylbenzo-thiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhyrazyl (DPPH) assay (Huang et al., 2005), and (b) hydrogen atom transfer ability as like oxygen radical absorbance capacity (ORAC) (Barba et al., 2014; [9], Lemus-Mondaca et al., 2016; Prior et al., 2005; Huang, Ou & Prior, 2005). Frequently, ascorbic acid is the reference antioxidant used. Needless to say, for comparison studies, the *Stevia* leaf extracts purity and composition ought to be taken note of (Myint et al., 2020). Shuckla *et al.* (2009) and Shuckla *et al.* (2011) reported similar antioxidant properties in *Stevia* leaf extracts to that of ascorbic acid. Ethanolic extract (61.50 mg GAE/g) and aqueous extract (56.73 mg GAE/g) of *Stevia* leaf extracts showed an antioxidant activity of up to 200 $\mu$g/mL dose dependently in a DPPH assay. The rate at which oxidation was inhibited in aqueous extract and ethanolic extract ranged 40.00-72.30% and 36.93-68.76%, respectively. The antioxidant reference (ascorbic acid) range was 64.26-82.58%. Ascorbic acid, aqueous leaf extract, and ethanolic leaf extract scored 26.75, 83.45, and 93.46 $\mu$g/mL $IC_{50}$ values, respectively, in a DPPH assay (Myint et al., 2020).

Antioxidant activity of ethanolic leaf extract against superoxide anions, nitric oxide, and hydroxyl radicals scored $IC_{50}$ values of 100.86 (81.08) $\mu$g/mL, 93.73 (132.05) $\mu$g/mL, and 100.86 (93.46) $\mu$g/mL, respectively. The ascorbic acid standard $IC_{50}$ values were observed to be 71.41, 66.01, and 26.75 $\mu$g/mL, respectively. Consequently, one can infer that Shuckla et al. (2009) and Shuckla et al. (2011) results do not agree with the notion that *Stevia* leaf extracts do exhibit similar antioxidant activities as ascorbic acid. Considering $IC_{50}$ values, ascorbic acid outweighed *Stevia* leaf extracts in terms of antioxidant activity. According to the Gawel-Beben et al. (2015) experiment, in which glycol-aqueous extracts, ethanolic, and aqueous extracts were analyzed for antioxidant activity against DPPH and ABTS radicals,

Table 2: Some of the free radicals' potential harm to biomolecules in the body.

| Species | Source | Reaction(s) with biomolecules |
|---|---|---|
| $O_2^{\cdot -}$ | Enzymatic process, autoxidation reaction, and nonenzymatic electron transfer reactions | It can act as reducing agent of iron complexes such as cytochrome-c or oxidizing agent to oxidize ascorbic acid and $\alpha$-tocopherol |
| $HO_2\cdot$ | Protonation of $O_2\cdot$ | Initiates fatty acid peroxidation |
| HO· | $H_2O_2$ generates HO· Through the metalcatalyzed Fenton reaction | HO· reacts with both organic and inorganic molecules including DNA, proteins, lipids, and carbohydrates |
| NO· | Action of nitric oxide synthase using arginine as a substrate and NADPH as an electron source | NO· is an intracellular second messenger stimulates guanylate cyclase and protein kinases and helps in smooth muscle relaxation in blood vessels |
| $NO_2\cdot$ | Protonation of ONOO· Or homolytic fragmentation of $ONOOCO_2\cdot$ | This radical acts on the antioxidative mechanism decreasing ascorbate and $\alpha$-tocopherol in plasma |
| ONOO· | Reaction of $O_2\cdot$ With NO. | ONOO. is a strong oxidizing and nitrating species of methionine and tyrosine residues in proteins and oxidizes DNA to form nitroguanine |
| $CO_3^{\cdot -}$ | The intermediate of reaction superoxide dismutase (SOD)-$Cu_2^{+}$-OH· react with bicarbonate to generates $CO_3^{\cdot -}$ | Oxidizes biomolecules such as proteins and nucleic acids |
| $ONOOCO_2^-$ | The peroxynytrite-$CO_2$ adduct is obtained by reaction of $ONOO^-$ with $CO_2$ | This anion promotes nitration of tyrosine fragments of the oxyhemoglobin via free radicals |

glycol-aqueous and ethanolic extracts exhibited the highest activity with the $IC_{50}$ values 0.38 and 0.71 $\mu$g flavonoids/mL and 2.08 $\mu$g flavonoids/mL. Ethanolic extracts showed the highest potency to chelate the ferrous ion (Myint et al., 2020). Generally, the higher the TPC, the stronger the antiradical activity. Nonetheless, one ought to bear in mind that the *Stevia* leaf extract antiradical activity is 'water-loving,' just like that of ascorbic acid. *Stevia* leaf extract is more thermostable than ascorbic acid (Myint et al., 2020).

In addition, antioxidant modes of action vary from one antioxidant to another. A number of such mechanisms are reported. Among such mechanisms are singlet oxygen quenching, potency to chelate metal ions, singlet electron transfer (SET), mechanisms that mitigate tocopherol radicals, hydrogen atom transfer (HAT) mechanisms, suppression of oxidases mechanisms, and some which activate antioxidant enzymes. There is a need, therefore, to carry out many assays for antioxidant activities (Myint et al., 2020; Santos-Sanchez *et al.* 2019). Many polyphenols found in *Stevia* are basically the same as those found in other plants. Antioxidant properties in *Stevia* are therefore expected. Yu et al. (2017) observed that a *Stevia* extract of total phenolic compound 71.46 mg GAE/g inhibited the formation of hydroperoxide in fish oil. According to Ortiz-Viedma et al. (2017), 23.97 mg GAE/g DW improved the shelf-life and quality of salmon paste by presenting a lipid oxidation inhibition activity and suppression of pathogenic microorganism development in the salmon paste that was kept under refrigeration conditions.

Notably, the geographical place from where the *Stevia* samples are taken affects the antioxidant activity. Also, different parts of the herb give different antioxidant activities. Leaves, stems, and roots, for example, do not produce antioxidants of same activity ([10]; Zayova *et al.*, 2013). Singh and colleagues observed that the highest ABTS radical scavenging activity was scored by a methanolic root extract (64.23 mM). The flower, stem, and leaf extracts showed 46.49, 49.28, and 56.26 mM ABTS radical scavenging activity (Myint et al., 2020).

Unfortunately, antioxidant assays done *in vitro* do not represent the true *in vivo* biochemical reactions. One of the reasons is that free radicals easily spread. Also, majority of free radicals have a short life span. As a result, the chances of an antioxidant capturing the radical(s) are very slim. Worse still, the free radical-antioxidant reaction is a second-order reaction. Consequently, factors like concentration of antioxidants and free radicals are not enough considerations. Further considerations like the medium in which the free radical-antioxidant reaction is occurring, reaction conditions and chemical structures of both reactants need to be assessed too (Santos-Sanchez et al., 2019).

The reaction mechanism of phenolic compounds with the peroxy radical involves a proton transfer from phenol to the radical, leading to the formation of transition state H-O bond with one electron. If a phenolic antioxidant reaction occurs in an environment that favors the formation of hydrogen bonds, the antioxidant capacity is reduced. Alcohols were observed to have a dual effect on the reaction rate between peroxy radical and phenol. On one end of the spectrum, alcohols accept hydrogen bonds. On the other end, alcohols make a conducive environment for the phenol's ionization, forming phenoxide ions. The phenoxide ions formed quickly react with the peroxy radicals via an electron transfer mechanism (Santos-Sanchez et al., 2019).

Leopoldini et al. (2004) determined a theoretical dissociation bond energy of the O-H bonds in several phenolic compounds and their adiabatic ionization potentials. The compounds possessed different structures and polarities. The team simulated solvated and vacuum conditions. Individual compounds exhibited different mechanisms and reaction rates. Among these compounds were tyrosol, caffeic acids, hydroxytyrosol, and gallic acids. The compounds that exhibited hydrogen electron transfer (HAT) mechanism

were tocopherol, followed by hydroxytyrosol, gallic acid, caffeic acids, and epicatechin. Kaempferol and resveratrol exhibited a singlet electron transfer (SET) mechanism (Santos-Sanchez et al., 2019).

*4.2. Anti-Inflammatory Activities.* The body undergoes a natural response whenever foreign bodies invade or when injuries occur. Such a response is carried out by the immune system. Inflammation, however, is the cause of a number of serious diseases (Zou et al.,2020). Inflammation mobilizes immune cells to attack the inflammation rendezvous by producing special chemicals known as proinflammatory mediators (Al-Kharashi, 2018). Such mediators include interleukins (ILs). Interleukins are known for recruiting immune cells to aid warring against cancer, pathogens, and foreign bodies. Nonetheless, overproduction of the proinflammatory mediators is capable of inducing several acute and chronic ailments like arthritis, inflammatory bowel disease, and atherosclerosis [11–13]. The most commonly used anti-inflammatory drugs come with serious side effects like gastrointestinal toxicity, addiction, and drug tolerance. Therefore, a search for natural resources which exhibit anti-inflammatory activities with ideally no toxicity is on [14].

Natural product development like steviol glycosides, with an intention to prevent proinflammation is regarded as an efficient, safe, and cost-effective pharmaceutical invention (Auyeng et al., 2016; [15]). Many studies showed that *Stevia* extracts have anti-inflammatory properties. Among those studies, a few of them reported that hydroalcohol *Stevia* extracts (500 mg/kg) mitigated oxidative damage in the liver by changing levels of cytokines like TNF-$\alpha$, IL-6, and IL-1$\beta$. Also, the extract exhibited the ability to stop inflammation (Holvoet et al., 2015; Latha *et al*., 2017).

Cytokines are signaling molecules that are secreted during pathological and physiological processes by the immune cells such as lymphocytes, macrophages, and various stroma cells. Stevioside is capable of restraining the secretion of proinflammatory cytokines in macrophages following being challenged with lipopolysaccharides in a dose-dependent manner [16]. Meng et al. [17] also had similar observations that 200 $\mu$M stevioside restrained an inflammatory response that was induced by titanium particles in bone marrow-derived macrophages. In addition to that, stevioside prevented osteolysis in titanium particles-treated mice with doses of 10 or 30 mg/kg (Zou et al., 2020).

Basically, stevioside exhibits anti-inflammatory properties by downregulating the major two pathways, namely, nuclear factor kappa B (NF-$\kappa$B) and mitogen-activated protein kinase (MAPK) signaling pathways [17]. Mitigation of inflammation in a mouse that was treated infected with *Staphylococcus aureus* (*S. aureus*) was reported by Wang et al. [18]. The downregulation of MAPK and NF-$\kappa$B pathways' mechanism was exhibited in the mouse. Wang and colleagues started by conducting an experiment on primary mouse mammary epithelial cells (MMECs) that were infected with *S. aureus*. They noted that stevioside inhibited the production of cytokines like IL-6, IL-1$\beta$, and TNF-$\alpha$. Stevioside also prevented necrosis of the *S. aureus*-infected MMECs by downregulating the gene expression for type 2 Toll-like receptors (TLRs) (Zou et al., 2020).

Wang and colleagues advanced their research. They administered the *S. aureus* in the mouse's mammary gland intraperitoneally. The team observed that stevioside reduced inflammatory cell infiltration and maintained the histological mammary gland structure. Exact findings were noted when the mammary gland primary cultures were used for the experiment in lieu of the whole mouse. The cells were infected with *S. aureus*; however, stevioside protected the cells from death and inflammatory hazards by controlling the actions of TLR2 expression, NF-$\kappa$B, MAPK, and cytokines (Zou et al., 2020).

Reduction of both inflammation and oxidative stress by stevioside is reported by a number of scientists. Many mechanisms had been reported. One of the mechanisms by which oxidative stress is quenched is activating nuclear factor erythroid 2-related factor 2 (Nrf2). The protein, Nrf2, is capable of preventing inflammation process. Casas-Grajales (2019) observed that rebaudioside A inhibited the expression of the Nrf2. It also downregulated the expression of proinflammatory genes like MMP-13 protein, Smad7, NF-$\kappa$B, and TGF-$\beta$1 (Zou et al., 2020).

The notion that there is communication between Nrf2 antioxidant response and NF-$\kappa$B-inflammatory response had been reported (Li et al., 2008; [19]). Substances that activate Nrf2 are capable of inhibiting the phosphorylation of IKK and p65 NF-$\kappa$B subunit nuclear translocation, therefore preventing inflammation suppression (Zou et al., 2020).

*4.3. Antidiabetic Properties.* If insulin action, secretion, or both are not normal, one suffers from a group of carbohydrate metabolism disorders, namely, diabetes mellitus after some time. The ailments are chronic. Challenges like side effects rendered by diabetes mellitus therapeutic drugs make them less likely the favorable solution. Also, a few individuals can afford to buy such drugs. Therefore, an alternative way which is cheaper, effective, and affordable is needed. Many folks resort to the traditional herbs. Among those traditional plants is Stevia rebaudiana [20].

Ahmad and Ahmad [20] designed an experiment to examine the antidiabetic properties of aqueous extract of *Stevia rebaudiana* Bertoni leaves in streptozotocin- (STZ-) induced diabetes in albino rats. The duo injected 40 mg/kg body weight STZ intraperitoneally. The STZ was prepared in a citrate buffer (0.1 M, pH 4.5). The scientists injected STZ into the femoral vein of rats following an overnight fast. Initial drug-induced hypoglycemia mortality was circumvented by the administration of 20% glucose solution to the rats for twenty-four hours. The rats that served as normal during the experiment course were given standard diet and distilled water.

The biochemical serum at the end of the experiment was then analyzed for liver glycogen, glycosylated hemoglobin (HbA1c), insulin levels, and random and fasting blood glucose levels. Blood glucose levels were quantified using the GOD PAP Enzymatic Calorimetric Test Method according to Trinder (1969). The method reported by Nayak and Pattabiraman (1981) was used to quantify HbA1c levels in the

rats' serum. Liver glycogen levels were determined according to Babu et al.'s (2003) protocol. Finally, insulin levels were determined by ELISA (enzyme-linked immunosorbent assay) using the Boehringer-Mannheim kit (Andersen et al., 1993).

Ahmad and Ahmad [20] observed that administration of aqueous *Stevia* extract orally at different concentrations (200, 300, 400, and 500 mg/kg) for a period of 8 weeks markedly reduced the feed and water intakes of the diabetic albino rats. One can then infer that *Stevia* reduces the water and feed intake; therefore, weight gain is prohibited. This is simply because *Stevia* does not induce appetite (Robarts & Wright, 2010). The same results were reported for female Wistar strain rats at doses of 25, 250, 500, and 1000 mg/kg body weight (Abo et al., 2010).

The results reported by Ahmad and Ahmad [20] showed that *Stevia rebaudiana* leaf aqueous extract produced a significant dose-dependent reduction in body weight and body weight gain percentage of rats treated with the *Stevia* extract. The reduction in blood glucose levels is explained by the stevioside present in the extract. The rats lost weight probably because the diet glucose metabolism reduced or maybe the rats decreased rate of food consumption [21]. Reduction in weight of rats that were administered *Stevia* extract might be due to the large amount of stevioside (Awney et al., 2010). The finding is supported by a number of previous research studies (Bernal et al., 2011; Abd-El-Razek & Massoud, 2012; [22]).

Not only that, Ahmad and Ahmad [20] observed that a spectrum of different aqueous *Stevia* extract controlled random and fasting glucose levels with a significant good efficacy. It was observed that stevioside capably controlled glucose levels by enhancing insulin secretion, insulin sensitivity, and insulin utilization in insulin-deficient rats due to downregulation in phosphoenolpyruvate carboxykinase (PEPCK) gene in a rat liver [23]. *Stevia* is thought to contain biomolecules that are able to make the insulin receptor more sensitive to insulin or stimulate $\beta$-cells of islets of Langerhans leading to the insulin secretion. This favors biochemical reactions like glycolysis and glycogenesis that aid in lowering blood glucose levels ([1]; Abo Elnag *et al*., 2016; Awney *et al*., 2010).

The HbA1c levels of the rats treated with aqueous *Stevia* extract were almost normal ($\geq$6.5%, 48 mmol/mol). The explanation is that glycemic control mechanisms were mobilized to do the work successfully. The results found by Ahmad and Ahmad [20] are in agreement with Prasal et al. and Rao et al. (Prasad *et al*., 2016; Rao & Najam 2016).

The serum insulin levels in control rates were found to decrease because of the STZ injected. Consequently, $\beta$ cells decreased, causing insulin secretion to fall as well. Insufficient insulin secretion causes hyperglycemia. Once hyperglycemia occurs, oxidative damage ensues due to ROS and diabetic complication development (Kangralkar et al., 2010; [20]). When the diabetic albino rats were given different concentrations of aqueous *Stevia* extracts, a significant improvement in insulin levels was noted. Stevioside inhibited hepatic PEPCK and gluconeogenesis and, at the same time, enhanced hepatic glycogenesis that led to insulin sensitivity and insulin secretion (Yang et al., 2009). Stevioside in the extract acted upon pancreatic tissue, exerting a beneficial antihyperglycemic effect through the PPAR$\gamma$-dependent mechanism. Other researchers reported the same mechanism ([22]; Akbarzadeh *et al*., 2015).

Stevioside is capable of inhibiting gluconeogenesis simply by inhibiting the glucagon action. Steviol glycosides modulate pancreatic beta cell function by enhancing TRPM5 (transient receptor potential cation channel subfamily M member 5). TRPM5 is an activated calcium cation channel protein that is expressed on beta cells and gut peripheral enteroendocrine cells by speeding up the rate of insulin production in response to glucose stimulation. One should bear in mind that rebaudioside, stevioside, and steviol are not the ones that have a direct interaction with the TRPM5 but the steviol moiety (Scaria et al., 2017; Prata *et al*., 2017). Steviol glycosides have the capability to act as the insulin receptor ligands (IR or IGF-IR) potentiating the P13k/Akt pathway. The biochemical pathway results in the translocation of glucose transporter 4 (GLUT-4) from an intracellular pool to the plasma membrane. Consequently, glucose enters into the cells and thus mimics the action of insulin (Fasiha et al., 2020).

*4.4. Antihyperlipidemic and Hypotensive Effect.* Aqueous *Stevia rebaudiana* extracts decrease fatty acid and cholesterol synthesis, mitigating LDL, total cholesterol, and triglycerides. Simultaneously, the extracts elevate HDL cholesterol (Scaria et al., 2017). The mechanism by which *Stevia leaves* aid in controlling blood pressure is relaxation of arteries and prevention of calcium accumulation on the arterial walls that favors vasodilation and reduction of total peripheral resistance and extracellular fluid volume. Hypolipidemia and hypotension causes a cardioprotective effect (Fasiha, Shahid & Faiz-ul-Hassan, 2020).

*4.5. Antitumor Effect.* Different types of cancers such as breast cancer, skin cancer, and ovarian cancer were found to be stopped and cured by stevioside. The stevioside induces apoptosis biochemical pathways. The cascade of the biochemical events leads to the upregulation of apoptotic proteins such as Bax, Bc1, and caspase-9. Cancer cell viability is mitigated by DNA synthesis inhibition and induced cell apoptosis. Stevioside metabolite, isosteviol, exhibited an inhibitory property against DNA topoisomerase II and DNA polymerase. The metabolite is capable of inactivating the P13/AKT signaling pathway by blocking P13 and AKT phosphorylation. There is an antimitotic compound extracted from *Stevia* called centaureidin. The compound has a potential use in tumor therapy (Mohammad, 2018; [24]; Fasiha, Shahid & Faiz-ul-Hassan, 2020).

*4.6. Antibacterial and Antifungal Activity.* Plant infections are a global challenge faced. Multidrug resistance (MDR) is rapidly skyrocketing due to an increase in number of microorganisms that resist a lot of drugs (Sundin & Bender, 1996). Dimaguga (1991) mentioned that scientists frequently screen for the active chemical components in herbs and plants. This is done to find solutions to a number of

microbial diseases and other diseases. *Stevia* is full of chemical components namely, flavonoids, phenols, and terpenes, which have the antimicrobial activity (Taware, 2010). Reports revealed that *Stevia* is capable of inhibiting microbial growth. Therefore, it had found many applications in wound healing and gum diseases. Diseases like yeast infections and recurring streptococcal infections can also be cured using *Stevia* [25].

Muradashvili et al. (2019) designed an experiment to probe the antibacterial and antifungal activities of the *Stevia* extract against microbials that cause plant diseases. The team extracted the desired phytochemicals using different solvents. Antimicrobial assay was investigated using the Agar-well bioassay (Lindsay, 1962). The team made each extract of 10 mg/mL final concentration. All extracts were then analyzed for antimicrobial activity by measuring the diameter of zone of inhibition using the disc diffusion method (Valgas, 2007). The detailed methodology is expounded in Muradashvili et al.'s (2019) article.

The team observed that chloroform and ethyl alcohol extracts had highest inhibitory effects on bacterial strains. Chloroform extract inhibited the growth of *Ralstonia solanacearum* culture by scoring 15 mm diameter zone of inhibition after 24 hours. Ethyl alcohol scored 18 mm and lastly acetone which scored 6 mm diameter zone of inhibition. Detailed results are available in Muradashvili et al.'s (2019) article. Nonetheless, the take home point is that *Stevia* has antibacterial and antifungal properties (Muradashvili et al., 2019).

*4.7. Hepaptoprotective Properties.* Das and Khathiriya [26] designed an experiment to examine the hepatoprotective properties of *Stevia rebaudiana*. The duo used thioacetamide (TAA) to induce hepatotoxicity in healthy Wister-Albino rats which weighed about 150-250 g. The duo then went on to evaluate 200 and 400 mg/kg/day doses on the rats following induced liver damage. An observation made concerning 400 mg/kg dose of aqueous *Stevia* extract administered orally was that it reduced levels of the liver enzymes like aspartate transaminase (AST, $131 \pm 3.0$ IU/L), alanine aminotransferase (ALT, $62.2 \pm 1.8$ IU/L), gamma glutamyl transpeptidase (GGT, $13.0 \pm 0.3$ IU/L), alkaline phosphatase (ALP, $197.4 \pm 3.2$ IU/L), and total bilirubin levels as compared to the controls used.

TAA has been commonly used to induce a variety of hepatoxicity ailments in experimental animals. Levels of hepatoxicity range from nodular cirrhosis, liver cell proliferation, parenchymal cell necrosis, and pseudolobule formation. A number of researches done reported that a single dose of TAA is capable of causing centrilobular hepatic necrosis. Chronic administration of the hepatotoxin gives rise to liver cirrhosis in the rats. The mechanism by which the hepatotoxin works is that it forms thioacetamide-5-oxide which causes changes cell permeability, increase in $Ca^{2+}$ intracellular concentration, increase in nuclear volume, and nucleoli enlargement. The TAA also inhibits mitochondrial activity leading to necrosis (Mitra et al., 1998; Fort *et al*., 2008; Ahmad et al., 2002).

A number of scientists suggested that part of hepatotoxicity caused by TAA is mediated via oxidative stress which is caused by the action of cytokines through lipid oxidation (So et al., 2002; Okuyama *et al*., 2004). Another suggested mechanism by which TAA causes liver damage is by reducing the antioxidant properties of the liver [27]. Cirrhosis induced by TAA in animal models resembles vital features of human diseases [28]. Transaminases are good biomarkers of hepatocellular damage. The enzymes levels are naturally higher in the intracellular compartments than in the counterpart compartments. Escalated levels in the serum indicate liver damage. In the Das and Khathiriya [26] study, TAA was observed to increase serum levels of AST, ALP, GGT, ALT, and total bilirubin. Pretreatment of the rats with the aqueous *Stevia* leaf extract reduced the abnormal serum levels of the above enzymes. Membrane integrity was under restoration. It is thought that flavonoids present in the *Stevia* are responsible for the hepatoprotective properties [26].

*4.8. Nephroprotective Properties. Stevia* is capable of carrying out inhibition of apoptosis, oxidative stress, and inflammation, hence exhibiting nephroprotective properties. Initial stages of diabetes mellitus are characterized by two major kidney complications, namely, glomerular hyperfiltration and renal hypertrophy. It is reported that a mechanism by which renal hypertrophy and glomerular hyperfiltration occur is production of transforming growth factor $\beta$ (TGF-$\beta$) by mesangial components and overproduction of free radicals after hyperglycemia. *Stevia* being able to carry out antioxidation activities is able to alleviate and treat the renal diseases. Glycosides do not only mitigate kidney-injury-related diabetes, but also cisplatin-induced nephrotoxicity (Fasiha, Shahid & Faiz-ul-Hassan, 2020). According to Ramos-Tovar et al. [29], aqueous extract of *Stevia* prevented liver cirrhosis in rats that was induced by administering carbon tetrachloride for 12 weeks, 3 times a weak giving a dose of 400 mg/kg i.p. The crew observed that chronic administration of carbon tetrachloride elevated nuclear factor kappa B (NF-$\kappa$B), proinflammatory cytokine production, and oxidative parameters such as lipid peroxidation whereas nuclear factor-E2-related factor 2 (Nrf2) and glutathione levels decreased. Hepatic stellate cell activation and profibrogenic mediator expression induction were reported. As a result, extracellular matrix production followed. The authors reported that aqueous stevia extract exhibited antifibrotic properties. The mechanism is not known but the authors suggest that it might be due to the extract's ability to block the profibrogenic signaling pathway.

*4.9. Cardioprotective Properties.* In developing countries, cardiovascular diseases and resultant cardiotherapeutic regimens are the major causes of deaths. A calcium channel blocker, diltiazem, is primarily used to treat hypertrophic cardiomyopathy, systemic hypertension, and supraventricular arrythmias. Stevioside, a chemical component found in *Stevia*, has a synergic pharmacological activity. Bhatt et al. [30] designed an experiment to evaluate the cardioprotective properties of stevioside and possible synergistic effects upon coadministration with diltiazem. The team made use of standard cardiotoxicity models to test the hypothesis: ischemia-reperfusion injury (IRI) and isoproterenol-induced myocardial infarction through Langendoff set up. Rats were

Table 3: Some of the *stevia* species and their properties.

| Species | Properties |
|---|---|
| *S. eupatoria* (Spreng.) wild | Analgesic, anti-inflammatory, antihypertensive. |
| *S. pilosa* Lag. | Antimalarial, antipyretic, cathartic, diuretic. |
| *S. cardiatica* Perkins | Heart diseases. |
| *S. lucida* Lag | To cure wounds, to soothe pains, rheumatism treatment, anti-inflammatory |
| *S. nepetifolia* HBK | Dysmenorrhea treatment. |
| *S. petiolata* (Cass) Sch. Bip. | Meat-flavouring agent |
| *S. salicifolia* Cav | Rheumatism treatment, cathartic, treatment for intestinal upset due to parasites, purgative. Treatment for fevers and colds. |

grouped randomly into control animals (normal: physiological saline and toxic: isoproterenol, 150 mg/kg, s.c., and IRI-induced normal control animals) and treatment groups (diltiazem: 17.5 mg/kg, p.o., stevioside: 100 and 200 mg/kg, p.o. and combination of groups). The animals (female Wistar rats) were sacrificed at the end of the treatment period and histopathological, electrocardiographic, and biochemical analyses were done.

Bhatt et al. [30] observed that pretreatment with the stevioside prevented cardiac biomarkers from leaking into the extracellular compartments hence normalizing perfusate and serum levels of CK-MB, ALT, AST, LDH, and CK-NAC enzymes. Antihyperlipidemic effects were observed on total cholesterol and triglycerides levels dose dependently. Stevioside also exhibited a protective action on SOD and catalase. A combination of stevioside and diltiazem (200 mg/kg and 17.5 mg/kg, respectively) was found to be more powerful in terms of pharmacodynamic response by significantly normalizing electrocardiographic parameters, myocardial histology, antioxidants levels, and biomarker levels.

## 5. Anticancer Properties

Martínez-Rojo et al. [31] conducted an experiment on *Stevia pilosa* and *Stevia eupatoria* methanolic extracts to observe their effects on prostate cancer cells. The team further probed the effects of each extract combined with enzalutamide. The team used 1000 $\mu$g/mL of each extract and 40 $\mu$M enzalutamide. The wound healing assay was performed for PC-3 cells treated with combinations of each extract and enzalutamide. It was observed that the combinations were stronger than extracts alone in increasing the inhibition closure of the wound. According to the authors, the extracts alone (concentration range 250 to 1000 $\mu$g/mL) have no cytotoxic effects on human fibroblastic cells. The finding is supported by Panagiotou et al. [32] who exposed peripheral blood mononuclear cells to *Stevia rebaudiana* extract. Howbeit, in Martínez-Rojo et al.'s [31] project, the *Stevia* extracts were able to retard PC-3 and LNCaP cancer cell viability at all concentrations assessed. The result is supported by López et al. [33] and Vaško et al. [34] when breast cancer (MCF-7 and MDA), colon cancer (Caco2 and HCT116), lung cancer (A-549), cervical cancer (He-La), and pancreatic cancer cells (MiaPaCa-2) were exposed to *Stevia rebaudiana* extracts.

Šic Žlabur et al. [35] identified luteolin in *Stevia rebaudiana*. The following year, Martínez-Rojo et al. reported that luteolin showed cellular arrest and could induce apoptosis in a broad spectrum of cancer cell lines like prostate cancer (PC-3), liver cancer (SMMC7721), colon cancer (COLO205), and cervical cancer (HeLa) (Lu, Li and Li, 2017). Quercetin, another compound present in the herb, is capable of retarding cell proliferation and viability in MCF-7 breast cancer cells. It is reported that the mechanism behind is apoptosis activation which is achieved through elevation of BAX (Bcl-2-associated X protein) and caspace-3 expression levels while lowering Bcl-2 expression. Also, quercetin activates necroptosis via elevation of receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and receptor-interacting serine/threonine-protein kinase 3 (RIPK3) expressions [4, 36]. Different compounds found in the herb seem to have different mechanisms of arresting cell viability and proliferation.

*5.1. Safety.* The paper highlights mainly the medicinal properties of the herb. Notwithstanding, there are documented papers that reveal toxicity of the herb. The acceptable daily intake of *Stevia* dry extract defined by the Scientific Committee on Food of the European Food Safety Authority and Food and Drug Administration is 4 mg/kg body mass. One animal study revealed that the herb has a potential to cause allergy. In addition, it was observed that the *Stevia* extract reduced fertility in rats by up to 21% as compared to control rats. It is reported that fertility remained reduced by 47% even following 50 to 60 days recovery time frame (Lohner et al., 2017). The rats that were treated with the *Stevia* extract were found to have a decrease in the relative weight of seminal vesicle and testis as well as a significant decrease in the number of spermatozoa stored (Mazzei-Planas & Kuc, 1968; Melis, 1999). One study revealed that steviol is a mutagen although there is no supporting evidence as of late ((Pezzuto et al., 1985). The primary evidence of *Stevia* safety is that since its use about 1500 years ago by the Paraguayans, there have been no reports of adverse effects. Also, the Japanese consumed the herb in large quantities and no reports are available on the herb toxicity [37]. Moreover, a number of studies that probed *Stevia* effects on the human body

revealed no toxicity (Roy et al., 2010; Nikiforov *et al*., 2013; Uçar *et al*., 2017) [38] are shown in Table 3 [39].

*5.2. Conclusion.* One can confidently infer that *Stevia rebaudiana* is a versatile useful herb in the world. The herb is capable of treating renal diseases, liver pathologies, diabetes, hyperglycemia, hypoglycemia, hypertension, tumors, microbial diseases, and obesity. Nonetheless, researches are still going on to understand fully the herb. Therefore, the conclusion made is not everything known about the herb. This work highlights chemical composition of *Stevia rebaudiana* and its medicinal properties like antioxidant, antidiabetic, anti-inflammatory, antihypoglycaemic, anticancer, renal-healing, cardiac-healing, antilipidemic, and hepatoprotective effects.

## Data Availability

The data supporting this review are from previously reported studies and datasets, which have been cited.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Supplementary Materials

Stevia extracts are obtained via a plethora of extraction techniques like solvent extraction, selective precipitation, supercritical fluids, and chromatographic adsorption among others. The extracts are then concentrated and purified using purification techniques like ion exchange chromatography and high-pressure liquid chromatography. The constituencies separated include amino acids, fats, mineral ions, terpenes, flavonoids, steviols, steviol glycosides, phenols, and polyphenols. Phenols and vitamins are antioxidants. These antioxidants play a crucial role as anticancer molecules. Anticancer properties were reported in a number of papers. The DNA synthesis of cancerous cells is lowered when subjected to Stevia extracts. Steviol glycosides have an anti-inflammatory property (proinflammatory factors production decreases) and steviosides have an antidiabetic (insulin sensitivity increased). Stevia extracts were reported to have antihyperlipidemic and hypotensive effects (fatty acid and cholesterol synthesis lowered; vascular diameter increased, respectively). Plant secondary metabolites in Stevia were found to have antibacterial and antifungal activities. Flavonoids in Stevia are thought to be responsible for hepatoprotective properties. *(Supplementary Materials)*

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