A glance on sweet shrub *Stevia rebaudiana* Bertoni

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The sweet shrub *Stevia rebaudiana* Bertoni has been used throughout the world as a noncaloric biosweetener owing to its two major thermostable phytoconstituents – namely, stevioside and rebaudioside – which have recently been added to the European Union list of permitted sweeteners. A number of countries across the globe, such as Japan, China, Malaysia, Taiwan, Australia, Korea, etc., have approved the use of *S. rebaudiana*-based sweeteners in foods and beverages. However, several studies on this ancient plant have revealed many of its pharmacological properties, such as anticancer, antihypertensive, antibacterial, etc., and thus *S. rebaudiana* ought to be called a medicinal plant. As expected, in recent years, researchers have directed the focus toward *S. rebaudiana* and have been patenting their inventions. A number of review articles have been published on *S. rebaudiana* in relation to different aspects, but no one has reported on their patents published. Hence, it has become necessary to provide the up-to-date and collective information on studies conducted and patents on *S. rebaudiana* and its metabolites with respect to their commercial applications. A good number of patents and research articles have been published on *S. rebaudiana*. These patents and research articles of interest were divided on the basis of their pharmacological activity and pharmaceutical application, described and discussed below in this review article. Furthermore, the yearwise distribution of patents was presented as bar diagram.

Keywords:
natural sweetener, neutraceutical, *Stevia rebaudiana*, stevioside

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Introduction

Stevia rebaudiana Bertoni, belonging to the family Asteraceae, is one of the renowned natural sweeteners used worldwide and is commonly known as sweet weed, meethipatti, or honey leaf. It has been reported to be 300 times sweeter compared with sugarcane, and the dry leaves of this plant are 30 times sweeter compared with sugar [1]. *S. rebaudiana*, indigenous to the northern part of South America, is a small perennial, growing up to 65–80 cm tall, and has sessile, oppositely organized, 2–3 cm-long leaves. The *Stevia* genus comprises at least 110 species. Although there could also be as several as 300 species, of which ~200 species of *Stevia* are native to South America, no other *Stevia* varieties have exhibited an equivalent intensity of sweetness as *S. rebaudiana* [2]. *Stevia* is a semihumid subtropical plant that can be grown effortlessly in well-drained red and sandy loam soil of pH varying from 6.5 to 7.5, like any other vegetable crop. Saline soils should be avoided to cultivate this plant [3].

Throughout the world, *Stevia* genus is known for its sweetening potential. However, the literature has revealed that only 18 species possess this characteristic, after screening of about 110 species [4]. This sweetening potential is attributed primarily to eight-kaurene glycosides present in its leaves – namely, dulcoside A, rebaudiosides A–E, steviolbioside, and stevioside – which produce the sweet taste [5]. These glycosides are mainly compounds of the diterpene derivative steviol [6]. It is noteworthy that stevioside is the major constituent (3–8% by weight of the dried leaves) [7]. Although *S. rebaudiana* has an important role as a natural sweetener, it has other uses as well. Therefore, in this review, we have summarized the extraction of the active constituents present in *S. rebaudiana*, different patented compositions of *S. rebaudiana* that can be useful as a sweetener, and eventually its commercial application.

Methods

A search was made using the Google database for data acquisition related to the sweetening potential of *S. rebaudiana*. All downloaded patents and articles on *S. rebaudiana* were subjected to preliminary screening and were selected on the basis of the pharmaceutical application of plant extract, its metabolite, and ample active constituents. Furthermore, the selected articles were reviewed stringently, followed by drafting, conception, and design of the manuscript.

Stevia varieties

To reap the maximum benefits from this multifunctional plant, most of the research groups started producing this plant asexually. In such an attempt, Marsolais *et al.* developed a new and distinctive variety of *Stevia* and coded as 'RSII94–751'. This variety showed superior advantages than that of parent plant:

(a) A high ratio of rebaudioside A to stevioside;
(b) A high ratio of rebaudioside A to rebaudioside C; and
(c) A high ratio of rebaudioside A to dulcoside A [8].

Furthermore, variety improvement was carried out by recurrent crossing and selection of original variety, and the obtained *Stevia* variety had significantly smaller quantities of rebaudioside A than stevioside. This attempt was to improve the stevioside content rather than the rebaudioside A. Dried leaf extract of this novel *Stevia* variety showed low rebaudioside A content and high stevioside content. Alternatively, stevioside sweetener with improved quality of sweetness is often obtained by treating the extract with enzymes [9].

Subsequently, Britos utilized controlled breeding program to produce a new and distinct variety of *Stevia* plant named 'AKH L4' having high rebaudioside A content [10]. Recently, Garnighian described a brand new and distinct asexually reproduced *S. rebaudiana* plant named 'T60' having high leaf concentration of rebaudioside A. However, its tall, bushy nature, ovate leaves, and its long stem stature distinguishes it from other varieties [11].

Extraction process

After knowing about the importance of *S. rebaudiana,* it had become necessary to isolate the glycoside present in it. In such an attempt, initially, one research group disclosed a process for the extraction of sweet compounds from *S. rebaudiana* Bertoni, using column extraction and membrane separation. The purpose of this invention was to augment the separation of sweet compounds, whereas minimizing the separation of undesirable bitter-tasting compounds [12]. Subsequently, Chinnamma *et al.* described a method for obtaining steviol from *Stevia* spp. and provided an effortless process to obtain white steviol [13].

In recent times, a simple extraction technique has been provided for preparing an organic-certifiable *S. rebaudiana* extract with high sweetness and optionally with antioxidant properties. The process involved the extraction of *S. rebaudiana* leaves that have been previously dried and ground with a hot solvent, causing dissolution of the sweetening compounds, which include different steviosides and rebaudioside A [14].

Separation and purification of rebaudioside

The quest to purify the isolated compounds leads to the development of chromatographic methods. In such an attempt, a different research group developed chromatographic separation techniques for isolating sweetening components using an appropriate elution solvent [14]. Structures of all glycosides isolated from *S. rebaudiana* are shown in Fig. 1.

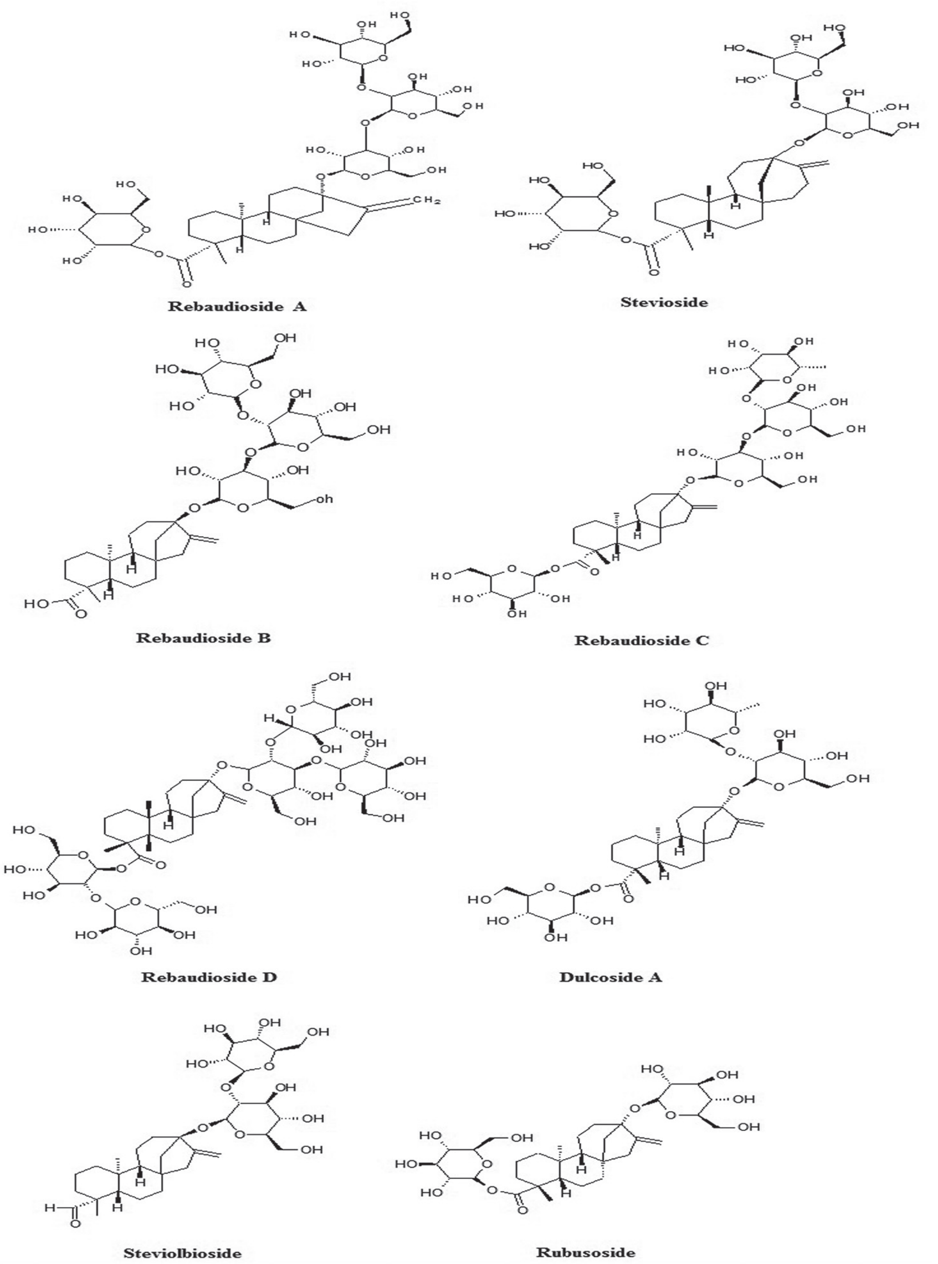
Few years ago, one research group disclosed methods for the chromatographic separation of rebaudioside A from glycoside solutions of stevioside that are obtained from *S. rebaudiana*. The method simply involved the utilization of an adsorbent comprising a polystyrene divinyl benzene support functionalized with tertiary amines, quaternary amines, or both, followed by contact of the adsorbent with the glycoside solution comprising rebaudioside A and stevioside for separating at least a portion of the rebaudioside A from the stevioside, thereby leading to the formation of a purified rebaudioside A solution [15].

Recently, Pnita *et al.* described a procedure for the purification of rebaudioside A (purity of 98.7%) from crude *Stevia* extracts using ethanol–water as solvent, followed by reflux, sonication, filtration, etc. [16]. An ecofriendly method for isolation of high-quality steviol glycosides, with improved final yield and improved organoleptic and biological antioxidant activity has also been disclosed and characterized using various chromatographic and analytical methods, including thin layer chromatography, nuclear magnetic resonance, Fourier transform infrared, ultraviolet spectroscopy, and high-performance liquid chromatography techniques [17–19]. The potential of rebaudioside M, isolated from *S. rebaudiana* Bertoni, as a high-potency sweetener, was examined with the Beidler model, which estimated that rebaudioside M is 200–350 times more potent compared with sucrose [20].

Separation and purification of stevioside

To extract, isolate, and purify stevioside, ample amount of efforts have been taken. In such an attempt, one research group extracted the dried leaves of *S. rebaudiana* with water, followed by lowering of pH up to 4 using tricarboxylic acid. After a few processes (neutralization with acid and treatment with water-immiscible solvent), isolated stevioside was purified by means of recrystallization with methanol. Thereafter, the isolated compound was compared with standard using Fourier transform infrared, nuclear magnetic resonance, and high-performance liquid chromatography studies [21]. To carry out isolation of steviosides with improved organoleptic activity, simple extraction and membrane purification processes were used. Extraction

Figure 1



Structure of isolated compounds from *Stevia rebaudiana*.

through pressurized hot water extractor, followed by purification and concentration of the sweet glycosides using ultra and nano (NF) membrane filtration, gave high-purity (98.2%) steviosides [22,23]. In another study, the ion exchange chromatography technique was used for purification of steviosides. Water extract of *S. rebaudiana* was purified by adding 5% $Ca(OH)_2$ three times. Obtained filtrates were collected and passed through ion exchange column (packed with glass wool) at a rate of 1 ml/s to remove the undesirable colors. The elution was isolated and concentrated using a rotary evaporator at 45°C to the maximum concentration value [24]. Repeated treatment of crude green extract with 10% activated charcoal and 5% celite followed by centrifugation at 12 000 rpm for 15 min gives a clear aqueous solution. Spray drying at optimized parameters was carried out for drying and getting a white stevioside powder [25].

Phytochemical studies

In an attempt to investigate the compounds mainly responsible for different activities of *S. rebaudiana*, *Stevia* leaf was analyzed for preliminary phytochemical studies to determine moisture content, total ash, water-soluble ash, sulphated ash, acid-insoluble ash, and extractive values in different solvents [26]. Quantitative investigation of biochemical content of various extracts of *S. rebaudiana* leaves showed that, in dry leaf extract, reducing sugars are present in highest percentage, followed by carbohydrates, proteins, and amino acids [27].

Application of *Stevia rebaudiana*

It is well known that *Stevia* is about 300 times sweeter compared with conventional sugar. Consequently, a lot of research work has been carried out utilizing its sweetening potential to develop novel compositions. Studies have reported the health-promoting applications of this magical natural herb *S. rebaudiana,* which is well-known as a therapeutic agent and an efficient medication for curing chronic diseases.

Sweetening and flavoring agent

People worldwide regularly customize the taste of food and beverages by adding sweetener for increasing its appeal. In such an attempt, Tezuka and colleagues prepared a formulation comprising a *Stevia* sweetener with a rebaudioside A (95% or greater) along with sugarcane-derived extract and g-aminobutyric acid. This superior formulation adequately reduced the aftersensation of sweetness and bitterness of high-purity rebaudioside A products, while providing exceptional sweetness and desirable flavor [28].

It is noteworthy that micronized *Stevia* compositions having particle sizes of less than about 20 mm were also found beneficial in decreasing the aftersensation related with typical *Stevia* compositions [29]. Furthermore, Galindo and colleagues described a sweetening composition comprising a mixture of *Stevia* extract or purified rebaudioside A and gymnemic acid in appropriate proportions. Surprisingly, this formulation reduced the aftersensation usually present in *S. rebaudiana* without affecting its sweetening intensity [30]. Similarly, Shimizu *et al.* described a sweetener that contains a *Stevia*-derived sweet substance and pulverized cyclodextrin of appropriate size [31]. Similarly, a liquid formulation comprising a minimum of one steviol glycoside (rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, dulcoside A, steviol, stevioside, and/or steviolbioside) dissolved in glycerol can be used as a food additive, a sweetener, and/or a dietary supplement [32].

The method of developing a better-tasting natural sweetener composition by removing or reducing volatile elements from dried vegetative matter (*Stevia* leaves with less than 9% moisture content) has been disclosed. This reduced water content from the surface of leaves leads to more water absorption during extraction step and ultimately facilitates the release of desirable flavor components from leaves to water. This modified vegetative matter showed a better-tasting flavor [33].

In another interesting work, Jingang *et al.* found that rebaudioside A do not have a uniform distribution or dispersion in aqueous solutions such as water. It associates together to form the clusters, thus causing impaired taste profile. To overcome this, they prepared micellar dispersions of a *Stevia* sweetener (e.g. one or more steviol glycosides) with one or more surfactants, which allow reduction in the steviol glycoside agglomeration, causing increased sweetness and/or decreased bitterness [34].

Catani described sweetener compositions including *S. rebaudiana* and simple sugars – that is, sucrose, fructose, glucose, or mixture thereof – as a bulking agent. The sweetener can be developed as non-free-flowing solids, granules, powders, or as liquids [35]. Similar to this, Purkayastha *et al.* also described a process for making a dust-free granulated sweetener having high solubility [36].

Walton *et al.* reported that rebaudioside-comprising compositions are appropriate for use as sweeteners in a variety of food and beverages. The sweetening composition is produced by subjecting *Stevia* extract to an acidic atmosphere under elevated temperature conditions. This treatment is sufficient to modify the glycoside composition of the extract, restrain bitter aftertastes, or offer a sweetening composition with a taste profile similar to that of sucrose sugar [37].

It is well known that rebaudioside A exhibits polymorphism, which is responsible for its low solubility. To overcome this, a method has been described for making extremely soluble individual or combined sweet glycosides from *S. rebaudiana* plant extract. The consequential sweetener promptly provides solutions with or up to or greater than 30% concentration, which are stable for more than 24 h. Spray drying of the highly stable concentrated solution gives a highly soluble *Stevia* sweetener powder [38].

Sweetening composition comprising *Stevia* and/or derivatives thereof and a compound sclareolide has also been reported. This invention conjointly provides a technique of reducing or masking the licorice aftertaste of *S. rebaudiana* [39,40]. Sweetening agents can be derived from *S. rebaudiana* and solids from *Aspalathus linearis* [41].

Maniga described a novel process that relates generally to a naturally preserved *S. rebaudiana* supplement (e.g. in liquid, beverage, or other form) that may have a flavor profile that masks the undesirable aftertaste, the undesirable smell of *S. rebaudiana,* or one that masks both. It also improved the shelf-life of naturally preserved *S. rebaudiana* supplement up to a minimum of 2 years without any artificial preservatives [42].

Additives

Improvisation in beverages with respect to flavor profiles, good taste, mouthfeel, etc. has become vital to meet changing market demands. In such an attempt, Lee described beverages and other beverage products having formulations appropriate to satisfy market demand for alternative nutritional characteristics or flavor profiles. This novel formulation comprised steviol glycoside and a berry component (berry juice, berry juice concentrate, berry juice extract, etc.) [43]. Furthermore, to improvise the taste of non-nutritive steviol glycoside sweetener in a beverage, beverage concentrate, or syrup, anisic acid was incorporated in a quantity sufficient to mask its metallic aftertaste [44]. Isomers of steviol glycosides were also found beneficial in beverage products and food [45]. Another interesting study described the compositions that can be used as bulking agents, sweeteners in foods and beverages.

The method for manufacturing food ingredients from *S. rebaudiana* Bertoni plant and their use in various food products and beverages has also been reported [46–48]. Recently, Leon delineated edible, natural, nontoxic, nonallergenic, digestible, food seasoner product for adding sweet flavor to foods [49].

Cosmetics

Application of care cream or makeup products to face involves rubbing for a better penetration, which may lead to unintentional spilling onto the lips, thus giving a very bitter taste. Masking of bitter substances has become a crucial step in cosmetic development. Utilization of an extract of *S. rebaudiana* along with a salt has emerged as an approach for masking the unpleasantness of bitter compounds present in cosmetic or dermatological formulations without changing the nature of the fragrance or the color of the formulation [50]. Recently, Goralczyk *et al.* described the use of an oral composition comprising steviol precursors, *S. rebaudiana* extract, or steviol, which surprisingly enhances the appearance of hair and counteracts hair loss. It further relates strategies of improving the appearance of hair by oral administration of an effective amount of *Stevia* extract, steviol precursors, or steviol [51].

Bioavailability enhancement

Approximately, more than 40% of newly developing drugs are poorly water soluble; thus improving its solubility is an enormous challenge to formulation scientists. The literature has revealed that various polymers have previously been used for improving the solubility of poorly soluble drugs.

In such an attempt, Gokaraju *et al.* described a composition consisting of an effectual amount of *S. rebaudiana* compound selected from raw powder or extract, fraction(s), compound(s), or mixtures derived from the entire plant or leaves of *S. rebaudiana* for enhancing the bioavailability and bioefficacy of therapeutic agents, supplements, cosmetic agents, beverages, and food ingredients selected from, although not restricted to, pharmaceutical drugs, proteins, minerals, micronutrients and macronutrients, enzymes, vitamins, amino acids, neutraceuticals, antioxidants, and herbal drugs/products [52].

Anticancer

The literature revealed the anticancer activity of *S. rebaudiana.* In such an attempt, the anticancer activity of ethanolic extract of leaves of *S. rebaudiana* has been determined in rats induced with Erlisch ascites carcinoma. The extract inhibited proliferation of cell count considerably ($P < 0.01$) compared with 5-fluorouracil [53]. Furthermore, the effects of stevioside on the cytotoxicity, induction of apoptosis, and also the supposed pathways of its action in human breast cancer cells (MCF-7) have also been demonstrated [54]. The toxicological effects of low concentrations of stevioside on apoptosis induced by serum deprivation using the PC12 cell system have also been evaluated [55].

Anticariogenic

S. rebaudiana presents properties that potentially are anticaries and thus it is useful in periodontal diseases. The literature has revealed that *Stevia* extracts have antibacterial activity on *Streptococcus mutans*, *Streptococcus sobrinus,* and *Lactobacillus acidophilus*, organisms that are closely related to the production and development of tooth decay [56].

Antihypertensive and heart tonic

Stevia exhibits vasodilatory actions in both normotensive and hypertensive animals [57,58]. Isosteviol is a derivative of stevioside, a constituent of *S. rebaudiana,* and has been examined for angiotensin-II-induced cell proliferation in rat aortic smooth muscle cells [59]. Several studies with rebaudiosideA on normotensive and hypotensive patients indicated that it has no effect on blood pressure [55,60,61]. It has been found that stevioside is a safe and effective compound in the treatment of hypertension and also has no adverse effect on sexual function [62,63].

Antidiabetic

Stevia has a potential role in abrogating insulin resistance and diabetes [64]. Only rebaudioside A indicates no effect on blood glucose [65]. However, it was found that the extracts of *S. rebaudiana* could decrease the blood glucose level in diabetic rats in a time-dependent manner [66]. It acts mainly by antagonizing the necrotic action of alloxan, causing revitalizing effect on the b-cells of the pancreas [67,68]. This property makes *S. rebaudiana* more effectual compared with *N*-nitro-l-arginine (l-NNA) [69]. It is worthy of mention that it could protect rats against streptozotocin-induced diabetes, remarkably decrease the threat of oxidative stress, and improve liver and kidney damage [70]. Similar to extract, powdered form of *S. rebaudiana* leaves also showed both hypoglycemic and body-weight-reducing effects in streptozotocin-induced diabetic rats when compared with glimepiride [71].

Antiobesity

S. rebaudiana extract is acceptable and its use can be beneficial to weight looser [72]. Very few studies are available under this area. One research group has demonstrated the potential of *S. rebaudiana* extract supplement as an antiobesity drug on high-fat-diet-induced obese mice [73].

Antioxidant

Oxidative stress could lead to numerous diseases such as cardiovascular disease, neural disorders, cancer, etc. [74]. Proper balance between oxidants and antioxidants is essential. Inulin and stevioside maintain this balance by means of superior scavenging of both hydroxyl and superoxide radicals compared with mannitol and sucrose due to the presence of greater amount of phenolic compounds in it [75,76]. Stevioside also acts as a promising potential therapeutic reagent against the lipopolysaccharide-induced acute lung injury because of its ability of inhibition of the nuclear factor-κB (NF-κB) signaling pathway [77]. It acts as a strong natural candidate, particularly for diseases that are caused because of free radicals [78].

Wound healer

The extract of the leaf of *S. rebaudiana* was found to be effective in the functional recovery of the wound healing in a dose-dependent manner [79].

Antifungal, antimicrobial, and larvicidal activity

Antituberculosis activity of the derivatives of glycoside steviolbioside from the plant *S. rebaudiana* and diterpenoid isosteviol containing hydrazone, hydrazide, and pyridinoyl moieties has been studied [80,81]. *S. rebaudiana* has been reported to contain antioxidant compounds that may have antifungal properties against *Aspergillus flavus* and *Fusarium verticillioides*. Hence, it could be developed as an alternative treatment to control aflatoxigenic mycobiota in moist maize [82]. It also exhibits larvicidal potential against *Anopheles stephensi* and can be further used for vector control alternative to synthetic insecticide. The crude extract of *S. rebaudiana* exhibits a wide spectrum of antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis, Aeromonas hydrophila, Vibrio cholera, and Salmonella typhi* compared with azithromycin and cepaxim as typical antibiotics [83–87].

Anti-inflammatory and immunomodulator activity

Stevioside acts as therapeutic agents against inflammatory diseases by inhibiting the activation of NF-κB and mitogen-activated protein kinase signaling and the release of proinflammatory cytokines [88,89]. Stevioside holds promise as an immunomodulating agent, which acts by stimulating both humoral and cellular immunity along with phagocytic function [90]. Two biological effects of steviol in the colon have been demonstrated for the first time: stimulation of Cl(-) secretion and attenuation of tumor necrosis factor-a-stimulated interleukin-8 production [91].

Brain tonic

Fowler and colleagues described a novel neutraceutical composition containing *S. rebaudiana* extract or its constituents, such as stevioside and steviol, as active ingredient(s). The compositions are helpful for improvement of psychological feature functions, such as learning, memory, alertness, and psychotic stability. It has the ability to inhibit glycine reuptake by inhibiting the glycine transporter, $GlyT_1$. The resulting increase in extracellular glycine levels leads to an increased activation of *N*-methyl-d-aspartate receptors, which is the first step toward inducing transcriptional activation of a variety of genes and subsequently to induce long-term potentiation, the main cellular mechanism concerned in memory formation and consolidation [92].

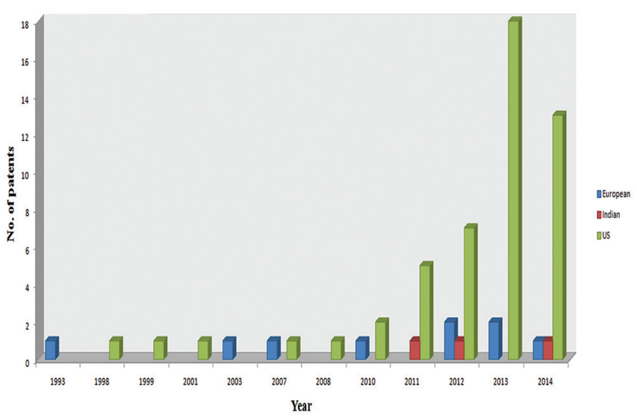
Toxicity and safety studies

As already discussed, *S. rebaudiana* is useful in a variety of diseased conditions. Keeping the advantages of *S. rebaudiana* in view, its toxicity and safety studies are needed to be investigated. The results of recent toxicological studies, along with the clinical studies in humans, demonstrate lack of pharmacological activity and reproductive toxicity, thus supporting the safety of high-purity (≥95%) rebaudioside A, stevioside, or steviol glycoside mixtures [93]. In a recent study, it was demonstrated that dietary administration of high concentrations of rebaudioside A for 90 consecutive days to Sprague–Dawley rats was not associated with any signs of toxicity [94]. The majority of the findings show that stevioside and steviol have no evidence of genotoxic activity [95]. It has been found that high purity rebaudioside A (rebiana) produced to food-grade specifications and according to Good Manufacturing Practices is safe for human consumption under its intended conditions of use as a general-purpose sweetener [96]. The yearwise distribution of patents filed is shown in Fig. 2.

Conclusion

In recent years, dietary and health demands have been increasing for natural non-nutritive high-intensity sweeteners with low-calorie value as a substitute to sucrose. Although ample components have been isolated from leaf extracts of the *S. rebaudiana*, the best known are diterpenoid glucosides involving stevioside (5–22%) and rebaudioside-A. These glycosides are 300 times sweeter compared with sugar and also exhibit wide therapeutic activity. Despite such high potential of steviosides, the conventional methods of isolation involve long extraction and purification procedures; therefore, optimization of product yields is a challenging problem. Hence, major focus should be directed towards developing novel methods for isolation of these glycosides. Compared with the last decade, now researchers have directed towards the widespread applications of *S. rebaudiana* as gradual increase in patents filed on *S. rebaudiana* Bertoni has been noted. This indicates the usefulness of *S. rebaudiana* for human beings. However, its use in obesity still lacks evidence; thus, further detailed investigation is needed.

Figure 2



Yearwise representation of patent filed on *Stevia rebaudiana*.

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Conflicts of interest

There are no conflicts of interest.

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