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Review Article

Review on phytochemicals and biological activities of natural sweeteners *Stevia rebaudiana* Bertoni

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Abstract: Diabetes is a chronic metabolic disease that creates high blood sugar level. Therefore, diabetes awareness is necessary to prevent diabetes by reducing sugar intake and using low-calorie alternative sweeteners instead. *Stevia rebaudiana* is a medicinal plant species belonging to the Compositae family. It is a sweet herb that contains diterpene glycosides, which are directly responsible for the sweet taste, but they have no caloric value. Since ancient times, there have been several reports on the use of *S. rebaudiana* as an alternative sweetener and extended research has been conducted on its phytochemicals and biological activities. The plant contains a good number of phytochemicals with significant biological activities, namely polyphenolic derivatives, diterpenes glycosides, alkaloids, glycosides, tannins, chlorophylls, carotenoids, etc. For industrial use, those phytochemicals could be extracted from the selected plant and used for the preparation of nutraceuticals and food additives. *S. rebaudiana* is a natural herb; therefore, it has fewer or minimal adverse effects on human health. The selected plant in various forms is used for the treatment of diabetes, colon cancer, obesity, cavities, and others. However, the literature review shows that the information on this plant and its uses is not systematic. The purpose of the present review is to explore the status of phytochemicals and biological activities of the selected plant for young researchers. Therefore, the updated data will help them to develop new nutraceuticals and food additives that could help in the production of pharmaceuticals to treat different ailments.

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1. INTRODUCTION

Plants have been the primary source of safe medicine to cure various ailments. Traditionally, plants have been used to treat diseases since ancient times due to various biological and pharmacological activities (Zarawska, 1975; Kakkar & Bais, 2014; Rana & Bais, 2014). Since ancient times, herbs have been used as vital primary medicines to treat various ailments by different communities all over the world, including the Sultanate of Oman (Tandon *et al.,* 2004).

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Several evidence-based reports have been published on the clinical trials as well as quality control of herbal medicines (Steven & Ehrlich, 2009). The biological activities of more than 250,000 plant species have been well-discussed (Schoonhoven, 1982). Based on recent estimates, there are approximately 500 000 phytochemicals, but only 30 000 have properly been examined regarding their biological activities (Bemays & Chapman, 1994). Plant phytochemicals have a habitual interest in agriculture and pharmaceutical sectors because of the growth of organic products from plant sources in the United States (Isman, 1994).

The anticancer drug Taxol is manufactured from plants. The increasing aspiration by the communities for alternative medicine has added motivation for scientists to advance research in this field (Klayman, 1985; Phillipson & Wright, 1991; Beutler *et al.,* 1995).

The use of medicinal plants was first reported in Mesopotamia in about 2600 BC.; however, the therapeutic potential of medicinal plants was not emphasized in this record. In Ancient Egypt "Ebers Papyrus" dating from 1550 BC explained more than 800 natural remedies including the use of plants and their extracts, animal parts, and various minerals. The Assyrians, who governed Mesopotamia at the beginning of the first millennium BC, significantly merged antique knowledge about herbal remedies to treat diseases. From about 1100 BC, the Chinese Materia Medica was broadly explained and documented herbalists were fully focusing on cataloguing and documenting the therapeutic effects of plant compounds that they isolated from the plants. During that time, Shennong Bencao Jing (100-200 AD) in China accumulated and added more than 400 plant-based herbal drugs in Materia Medica of Dioscorides (Unschuld, 1988; Tang & Eisenbrand, 1992).

The genus *Stevia* Cav. is considered a sweet herb and it originated in Brazil and Paraguay. At the beginning of the sixteenth century, the people of Europe learned first that the leaves of Stevia are approximately 20-30 times sweeter than sugar. Since then dried leaves have been stored and used in raw form instead of synthetic sugar. The growth rate of the *Stevia* is slow and it does not disturb or increase the blood sugar levels in the living organisms. Several reports showed that *Stevia* extracts do not have any significant neurological or urological side effects (Tanaka *et al.,* 1997). Recently, based on its health benefits, *Stevia* is commercially produced in several countries including the United States, the Republic of China, Portugal, Paraguay, Brazil, Thailand, Poland, the Netherlands, India, and Korea, but Japan is the largest consumer of *Stevia* as an artificial sweetener in the World. Due to the importance of the selected plant, the purpose of this present review is to raise awareness of the status of phytochemicals and biological activities for young researchers.

1.1. Plant Description

S. rebaudiana is a flowering plant that belongs to the genus *Stevia* and Compositae family. It has several common names such as candy leaf, sweet leaf or sugar leaf (Katayama *et al.,* 1976; Goyal *et al.,* 2010; Misra *et al.,* 2011). It is one of the 154 members of the genus of *Stevia*, but only two species are sweet to taste. It is native to the border areas of Brazil and Paraguay. However, some countries gave more effort to produce *Stevia* for commercial purposes. Due to its sweetness, nowadays, it is commercially cultivated in various countries, namely Paraguay, Argentina, Germany, Republic of China, United Kingdom, Japan, Spain, Republic of South Korea, Canada, Australia, Mexico, Belgium, USA, Brazil, Israel, Malaysia, Indonesia, Taiwan, Thailand, Tanzania, and India. Now the leading *Stevia* producing country is China, which commercializes its processed products as artificial sweeteners. Currently, Japan is considered a major importer market for *Stevia*. The plant grows well similar to other vegetable crops and normally it grows well in subtropical countries. Due to its importance, *Stevia* has become cash crop in many countries and its cultivation is closely monitored by Ministry of Agriculture while measuring various parameters such as height, leaf weight, growth, insects as well as the content of sweeteners in the plant. The plantation of *Stevia* is from February to March each year and

the plant is harvested in June or July. It is a flowering plant and the flowers become within 54-100 days (Goyal *et al.,* 2010; Misra *et al.,* 2011). The concentration of sweetening ingredients in this plant is known to increase with the maturity of the plant.

1.2. Morphology

S. rebaudiana is a medicinal plant belonging to the Compositae family. The plant is a small perennial tropical shrub height up to 30 cm (Madan *et al.,* 2010, Figure 1). A mature plant could reach a maximum of 80 cm in height with woody stems. The leaves are oppositely arranged and the surfaces of the leaves are slightly glandular and have two discrete sizes. The leaves are oval in shape and the size of the leaves varies with environmental conditions such as types of soil, irrigation process, sunlight, air quality, etc (Madan *et al.,* 2010). The leaves have a sweet taste that remains in the mouth for at least an hour.

Figure 1. Different parts of *S. rebaudiana*

Plant **Seeds** **Rhizome**

The plant has spirally arranged small white tubular flowers. The shape of the fruit is oval and there is one seed in the seed coat. The seeds are about 3 mm long (Figure 1). The rhizome of this plant itself is slightly branching into roots (Figure 1). The selected plant grows well if the weather conditions are about 80% humidity and the temperature is within the range of 20-29^{o}C (Carneiro, 1930; Robinson, 1930; Brandle *et al.,* 1998; Meireles *et al.,* 2006).

1.3. Taxonomy of the *Stevia* Genus

The genus *Stevia* contains more than 240 species. Among the Gulf countries, only Iran and Iraq have a few endemic species, but they are not sweet. The climate in most parts of Oman is not suitable to grow this plant, as an experiment, the selected plant was planted on Jabal Al Akhdar and the plant is still surviving. Therefore, the mountains in Oman can be used for growing the selected plant species. Among the species, humans used only forteen species of *S. rebaudiana* for artificial sugar (Meireles *et al.,* 2006). The taxonomy and the most common species of *Stevia* are presented in Table 1 and Table 2.

Table 1. Taxonomy and species of *Stevia*

Kingdom	Plantae
Subkingdom	Viridiplantae - green plants
Infrakingdom	Streptophyta - land plants
Superdivision	Embryophyta
Division	Vascular plants
Subdivision	Spermatophytina - seed plants
Class	Magnoliopsida
Superorder	Asteranae
Order	Asterales
Family	Asteraceae (Compositae)
Genus	*Stevia* Cav.

Table 2. Most common species of *Stevia*

- *Stevia anisostemma*
- *Stevia bertholdii*
- *Stevia crenata*
- *Stevia dianthoidea*
- *Stevia enigmatica*
- *Stevia eupatoria*
- *Stevia lemmonii*
- *Stevia micrantha*
- *Stevia ovata*
- *Stevia plummerae*
- *Stevia rebaudiana*
- *Stevia salicifolia*
- *Stevia serrata*
- *Stevia tunguraguensis*
- *Stevia viscida*

1.3. Traditional Use

S. rebaudiana is a medicinal plant and it is considered an alternative sugar instead of synthetic sweeteners. Therefore, the plant is used mainly to stabilize the blood glucose in living organisms. In addition, the leaves are used for the treatment of other diseases, namely diabetes, cardiac diseases, high blood pressure, and weight loss in various alternative traditional systems (Saravanan *et al.,* 2012; Mann *et al.,* 2014). In the beginning, Japan and Korea used this plant extracts for the substitution of sucrose, but their governments in 1991 banned the plant extracts due to some issues. However, in 1991 the US Food and Drug Administration (USFDA) removed the ban, but they have imposed some restrictions on extracts and prepared finished products from the leaves of *S. rebaudiana*. Based on the chemical ingredients, the UK government raised several questions regarding the safety of the use of plant extracts. Due to chemical ingredients, during that time it has generated lots of misunderstandings about the safe use of *Stevia* (Shivanna *et al.,* 2013). After a long discussion, in addition to supporting the use based on the research and comprehensive international response, the USFDA approved the use of the crude extract of *Stevia* leaves as a safe natural and non-nutritive sweetener (NNS) (Schoonhoven, 1982). The leave powders of *Stevia* are used to treat the regulation of the heart rate (Shivanna *et al.,* 2013; Siddique *et al.,* 2014). Currently, *Stevia* is used as an artificial sugar as it does not affect the blood sugar of diabetics' patients. The plant does not have any adverse effects on renal functions like synthetic sweeteners. Not only that, the plant itself and its extracts have significant biological activities like anti-fungal, antioxidant, antihypertensive and anti-

bacterial properties. Therefore, several previous reports supported that the parts of the plant could be used in herbal preparation for the treatment of diseases such as tonics for diabetic patients, daily usage products e.g. mouthwashes and toothpaste (Meireles *et al.,* 2006). As a tea the leaves of *Stevia* give significant relief to diarrhoea (Shivanna *et al.,* 2013).

1.5. *Stevia* Commercial Products

Currently, several commercial products from *Stevia* are available in the global market. Table 3 presents some of the available commercial products. According to the needs, the stevia products can be collected directly from the *Stevia* producing companies as well as from pharmacies. Nowadays, the products can be bought from companies or pharmacies through Internet sources.

Table 3. Different types of **c**ommercial products from *Stevia*

Product name	Companies name	Type of medicine
Stevia powder	Stevia LLC, USA	Crystals
Extract	Life Extension Foundation, USA	Powder
JAJ Stevioside	JAJ Group USA	Powder
Liquid extract of stevia	Baar Product, Inc, USA	Liquid
Stevia Dark Liquid	Stevia NOW	Liquid concentrate
Pure powder extract	Stevia NOW	Powder extract
Stevia crude extract	Stevia NOW	Tablets

2. PLANT PHYTOCHEMICAL

Until now a good number of studies have been conducted on the phytochemicals of *S. rebaudiana*. Most scientists claimed that several groups of phytochemicals are present in the leaves. They are mainly diterpenes glycosides; alkaloids, steroids, tannins, saponins, flavonoids, glycosides, sterol, triterpenes, and anthraquinones (Table 4). Our previous study and several other studies on *Stevia* also showed that the plant leaves contain alkaloids, steroids, tannins, saponins, flavonoids, glycoside, sterol, triterpenes, and anthraquinones including phlobatannins (Hutapea, 1997; Genus Jan, 2002). The plant leaves also contain other vital compounds such as vitamins, folic acid, amino acids, diterpenes glycosides, and derivatives of caffeic acids, nutrients, and several other minor compounds (Soejarto *et al.,* 1982; Barriocanal *et al.,* 2008; Kobus-Moryson and Gramza-Michałowska, 2015; Jan *et al.,* 2021; Lemus-Mondaca *et al.,* 2021). In addition, reports showed that the essential oil of the selected plant contains palmitic, stearic, palmitoleic, oleic, linolenic and linoleic acids (Soejarto *et al.,* 1982). The plant leaves were analyzed by using atomic absorption spectrophotometry (AAS), the plant leaves contain high content of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sulphur (S) and sodium (Na). However, metals such as iron (Fe), copper (Cu), cobalt (Co), manganese (Mg), zinc (Zn), selenium (Se) and molybdenum (Mo) are present in smaller amounts. Some research also mentioned that the percentage of essential oil in the leaves is high compared to other plants. The oil of the selected plant contains a high percentage of carbohydrates, ash and protein. The range of foreign matter (2.86%), total ash (2.9% w/w), and moisture content (9.47%) have also been estimated (Soejarto *et al.,* 1982). The leaves are sweeter than the other parts of this plant such as flowers, stems, seeds, and roots. Based on the previous report, the leaves of this plant are 150 times sweeter than synthetic sugar (Soejarto *et al.,* 1982). The leaves contain several diterpene derivatives that are responsible for the sweet taste especially steviol glycosides. It is also reported that the leaves contain a maximum amount of diterpene glycosides derivatives during the flowering time (Soejarto *et al.,* 1982).

Table 4. Group of phytochemicals in the *Stevia*

Groups	Methanol extract	Hexane extract	DCM extract	Ethyl acetate extract	Butanol extract	Water extract
Alkaloids	+	-	-	+	-	-
Steroids	+	+	-	+	-	+
Tannins	+	-	+	+	+	+
Saponins	+	-	-	+	+	+
Flavonoids	+	-	-	+	+	+
Glycosides	+	-	+	+	+	+
Sterols	+	+	-	-	-	-
Triterpenes	-	+	-	-	-	-
Anthraquinones	+	-	+	+	-	-

+ = Present and - =Absent

2.1. Cariogenic and Mutagenic Effects

Previously, Das *et al.* conducted one study to explore the side effects of the prolonged use of stevia products or chemical ingredients: stevioside and rebaudioside-A. However, they didn't find any side effects due to the use of stevia (Das *et al.,* 1992). Regarding the mutagenic effects, several studies have been conducted on stevia by several authors, but the results are contradictory. Therefore, based on the previous report, the FDA stopped listing this herb as a substitute for sugar until the safety issue is settled (FDA, 2019).

2.2. Human Studies

Since the old times, *S. rebaudiana* has been used a natural sweetener instead of synthetic sugar. However, there is a lack of information on a clinical study of the *Stevia* plant and the chemical ingredients in the plant. Glucose tolerance tests were conducted on the effects of administering *Stevia* extracts. Based on the glucose tolerance tests before and after taking the *Stevia* extract, the results showed that the stevia extracts increased glucose tolerance and decreased plasma glucose concentration (Curi *et al.,* 1986). Later, Jeppesen *et al.,* worked on the derivatives of diterpene steviol and stevioside and the results showed that both ingredients can affect directly the pancreas for releasing insulin. His group concluded that the stevia plant could be useful to manage type 2 diabetes (Jeppesen *et al.,* 2000).

2.3. Total Phenols Content

Most of the researchers estimated the total phenol content of *S. rebaudiana* extracts with the modified FCR (Folin-Ciocalteu reagent) method (Amri & Hossain, 2018; Maisa *et al.,* 2021; Hossain *et al.,* 2022). The samples were analysed against the gallic acid standard and measured the absorbance by the UV-visible spectrophotometer method at the wavelength of 760 nm. The literature showed that most of the extracts from the *Stevia* gave a significant amount of total phenol content ranging from 7 to 15 mg/100g in the powder samples. The ingredients belonging to phenol groups are biologically active ingredients and they might be used for the prevention and treatment of diseases. Plant-drive phenolic compounds are widely called antioxidants (Amri & Hossain, 2018). The phenolic ingredients can stop the free radical reactions with other compounds in the human body. As a result, the phenolic compounds can prevent DNA damage and chronic health effects.

2.4. Total Flavonoid Content

The estimation of the total flavonoid content of varied polarities extracts of *S. rebaudiana* was conducted by the modified $AlCl_3$ method against quercetin standard as described by several authors (Al-Jadidi & Hossain, 2016; Al-Oraimi & Hossain, 2016; Al-Saeedi *et al.,* 2016).

Different polarities of plant extracts were analysed and the absorbance was measured by using the UV-visible spectrophotometric method at the wavelength 415 nm. The literature showed that among the six extracts e.g., methanol, hexane, ethyl acetate, chloroform, butanol and water extracts, the ethyl acetate extract gave the highest total phenol content ranging from 3 to 9 mg/100g powder samples (Al-Jadidi & Hossain, 2016). All flavonoid ingredients belong to phenol groups and they are biologically active and they can be used for the prevention and treatment of diseases. Plant-ingredient flavonoids contain compounds that are quite stable and they can also protect the human body from different cell reactions.

3. BIOLOGICAL ACTIVITIES

Since the old times, scientists have been paying attention to the biological activities of the plant extracts and confirmed their *biological activities such as antioxidant, antibacterial, antifungal,* antidiabetics, antihypertensive, cytotoxic, anti-inflammatory activities etc. *S. rebaudiana* gave significant biological activities. The literature search showed that plant extracts with different polarities displayed varied activities due to the chemical ingredients present in the extract. Biological activities play a vital role in the treatment of different diseases. The selected plant species showed varied biological activities; therefore, the selected species is used to treat various diseases including diabetes.

3.1. Antioxidant Activity

In general, the antioxidant activity of plant extracts was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) which was described by most of the authors (Al-Habsi & Hossain, 2018; Abdulsattar & Hossain, 2020; Al-Rashdi *et al.,* 2021; Al-Qassabi *et al.,* 2018; Hossain *et al.,* 2019). The same methods have been used to determine the antioxidant activity of various *S. rebaudiana* extracts and showed that the plant extracts with varied polarities gave varied antioxidant activity. The majority of the previous work showed that in the case of most plant species, the polar extracts have more significant activity than non-polar extracts (Al-Jadidi & Hossain, 2016; Saravanan *et al.,* 2012). However, some authors, found the reverse results meaning that non-polar extracts showed higher activity compared to polar extracts. Nevertheless, the total average antioxidant activities are quite high about 89% DPPH inhibition against ascorbic acid either in the case of polar or non-polar extracts (Shivanna *et al.,* 2014). In conclusion, the consumption of food with high antioxidants could reduce the risk of chronic diseases, including cardiovascular disease, colon cancers, diabetes, hypertension, etc. Free radicals from antioxidants are the vital source to prevent or reduce human cell damage through oxidation. The mechanism of preventing human cell damage by antioxidants is still.

3.2. Antimicrobial Activity

Still now, the researchers are using *in-vitro* and *in-vivo* studies to determine the antimicrobial activity of plant crude extracts (Al-Jabri & Hossain, 2018; Weli *et al.,* 2020; Al-Saeghi *et al.,* 2022). For the screening of the plant extracts, the scientists use the *in-vitro* method, however for clinical trials scientists use the *in-vivo* model. Scientists studied the selected *S. rebaudiana* extracts and found the best extracts that could be used to treat all kinds of infectious diseases without adverse effects (Curi *et al.,* 1986; Maisa *et al.,* 2021). The best extract with significant antimicrobial activity was found in hexane extract against Gram (+) bacterial strains within the range of 0-13 mm (Al-Jadidi & Hossain, 2016). However, other various polarities extracts from the leaves of *S. rebaudiana* also gave activity against all other Gram (+and -) with the range of 0-10 mm. The previous studies showed that all varied polarity extracts from this plant species gave moderate activity against the Gram (+ and -) bacterial strains. Based on the antimicrobial activity among the extracts, the hexane extract could be used as an alternative natural safe antibiotic instead of a synthetic antibiotic.

3.3. Anti-diabetic Activity

Diabetes is the deficiency of insulin. Most of the vital organs were affected due to diabetics. Scientists are looking for a natural safe remedy to treat or manage diabetes. *In-vitro* and *in-vivo* antidiabetic activity of the plant extracts was determined by using α-amylase inhibitory and α-glucosidase inhibitory bioassay as described by several authors (Ahmad & Ahmad, 2018; Dhasarathan & Theriappan, 2011). The same therapeutic approaches were used for the screening of the crude extracts of the selected plant species for their antidiabetic activity. The inhibition results obtained from both approaches showed that the stevia extracts significantly reduced postprandial elevation of blood glucose. Previous studies showed that all fresh extracts of the selected plant inhibit the α-amylase with an IC_{50} value of 2.679 µg/ml, which was higher than the standard acarbose (1.736 µg/mL). However, in the case of all commercial and wild samples, the inhibition capacity towards α-amylase deteriorated once the maximum capacity was reached, which was probably due to the saturation of the enzyme. The inhibition could be due to the ingredients that inhibit the α-amylase enzyme.

4. CONCLUSION

S. rebaudiana is a flowering plant that belongs to the genus *Stevia*. The selected plant is used mainly to stabilize the blood glucose in living organisms. In addition, the leaves are used for the treatment of other diseases, namely diabetes, cardiac diseases, control of blood pressure and weight loss in various alternative traditional systems. The focus of this present review is on the phytochemicals, pharmacological and toxicological activities status of the plant species. The plant can be used as a natural antioxidant and antibiotics as supplements and drugs. Therefore, further clinical and pathological studies must be conducted to investigate the unexploited potentials of the *Stevia* plant before using the plant as a drug to treat diseases.

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Declaration of Conflicting Interests and Ethics

The authors declare that in this review there is no conflict of interest. This study complies with research and publishing ethics. The authors are responsible for scientific and legal responsibility for manuscripts published in IJSM.

Authorship Contribution Statement

Amzad Hossain: Conceptualization; Data curation; Data analysis; Wrote a first draft of the review. **Said Al Harthy:** Literature survey; Data collection; Edit data. **Salem Said Jaroof Al-Touby:** Reviewing and Editing. **Yahya Bin Abdullah Alrashdi:** Reviewing and Editing

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