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REVIEW ARTICLE

Phytochemicals and Medicinal Properties of *Stevia rebaudiana*: A Review

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ABSTRACT
Stevia is used as an ayurvedic diet supplement because it is the most potential in Brazil, China agronomic budget. The dry leaves of Stevia rebaudiana (Asteraceae) are dealing out to produce Steviol and Stevioside as an identified natural sugar that is used in pharmaceutics, cosmetics, and food productions. Newly, reports about the therapeutic effect of stevia rise its significance in the globes. The yearly harvesting yield of stevia is valued approx 600 tons yearly (China harvests 75% of total) globally, and also stevia is reflected to be the most effective and valuable as sugar substitute for diabetic patients in the world; due to this there are chance of artificial production or defraud. Due to this maintenance of the quality of stevia and requirements of a certification by following GRAS, ISO, or the Food and Drug Administration (FDA) criteria and standards. In this review, the current (or sometimes less documented) information on Pharmacognosy, pharmacology, and ordinary approaches for quality estimate of stevia, as a therapeutic nutrition herbal tea, from field cultivation to market are reviewed.
Keywords*: Stevia rebaudiana, Asteraceae, phytochemistry, stevia, standardization, steviosides, rebaudioside- A.*

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INTRODUCTION

Stevia rebaudiana is an herbaceous, semi-bushy, perennial shrub (Asteraceae family) and it ia also well acknowledged as sweet leaf, honey leaf, candy leaf, sweet weed, or sweet herbs Natural Sweetener in the world [1]. Gaurani Indians widely recycled this shrub since 1500 years in South America (Paraguay, Brazil), who called it ka'ahe'e ("sweet herb") [2], the genus was termed for Spanish botanist and physician PetrusJacobus Stevus, a professor of botany at the University of Valencia [3]. Dr. Moises Santiago Bertoni is first coin the word stevia and discovered this shrub in 1888 at Paraguay. Paraguayan chemist Dr. Rebaudi in 1905, the plant was systematically called as S. rebaudiana. In 12/2008, the FDA provided a "no objection" authorization for GRAS status to Truvia which is industrialized by Cargill and Coca-Cola Company [4], it means that the pure stevia as plant or shrub is not safe for use in the dietary supplement but a highly purified product of this plantsis used as a safe dietary supplement [5]. In 2017, great pureness stevia glycosides are deliberated non-toxic and allowable as an ingredient in food products sold within the United State [6]. The outstanding features of the stem of stevia sweet within the taste which don't contain active compounds usually aren't collected. The leave of the stevia with carbohydrate-based compounds that's 2-3 hundred times sweeter than sugar. It is found that stevia contains alkaloid such as steviosides which shows insulin-tropic properties in pancreatic beta cells. It reduces the blood sugar level by increasing the insulin secretion. It is used as substitute sweeteners for sugars [7]. Currently, average yields reported fall between 2494-3628 kg per acre with the plant production within the lower range. the wealth for conservative manufacture is about $1.254 per kg and herb production is $0.75 per pound in New Bern, NC [8].

CLASSIFICATION

Traditional Usage:

Stevia has been used in dietary supplements as a non-caloric Sweetener [9]. Beside through sugariness, an unpleasant taste is also identified in humans [10]herbal tea, Natural sugar, and Ayurvedic health system as Anti-diabetic, Anti-obesity, and Anti-cholesterol. Many pasts on the usage of S. rebaudiana are given by the antediluvian Ayurvedic system of medicine [11]. S. rebaudiana leaves has been endorsed as a cure in contradiction of several prolonged and non-chronic diseases like renal disease, diabetes, cancer, obesity, inflammatory bowel disease, cardiovascular disease, and dental caries.

Common Name of *Stevia rebaudiana*:

Stevia, candy leaf sweet leaf of Paraguay, sweet-herb, honey yerba, honey leaf, yaawaan,

Vernacular Names

Hindi: meethipatti
English: Sweetleaf, Honey leaf, Sweet herb
French: Stévia or Stévie
Marathi: MadhuParani
Sanskrit: MadhuPatra
Tamil: SeeniTulsi
Telugu: MadhuPatri

Taxonomical classification:

Kingdom: Angiospermae
Class: Dicotyledons
Group: Monochlamydae
Order: Asterales
Family: Asteraceae
Subfamily: Asteroideae
Tribe: Eupatorieae
Genus: Stevia
Species: rebaudiana

Plant description

Macroscopically Characteristics:

Stevia iscultivating up to 1m tall and has leaves 2-3 cm long. Macroscopic character expressed by Fig 1

Leaves –Sessile Green in color.
Odour- Odourless.
Taste- sweetish
Size- 5 cm in length and 3 cm in width
Shape- ovate
Extra features- leaves acuminate petiolate, faces are glabrous
Flower- white, throats funnel form lobes [12].

Climate and Land required for Cultivation:

S. rebaudiana is that the best remedial food plant, as a source of stevia, which has highest exporting status in china approx. 75% of the export of stevia from china that's by china is the biggest exporter in the world and Paraguay, Central America, Korea, and Thailand. The cultivated land area of stevia in all over the world is 32000 hectares. Notwithstanding china is the best region for development, Central America and Brazil are the appropriate areas for the cultivation of stevia. Thailand Korea and India are the most suitable country for the cultivation of stevia. In Stevia may be a perennial herb natural to between 22°-24° south and 53°-56° west in Paraguay and Brazil [13], [14]. stevia developed at higher latitudes even have a better proportion of sugary glycosides [15]. Nourishment and climate show vital roles in the expansion and secondary metabolites of stevia plants [16]. At low temperature (below 20 ºC) and day size is smaller amount then 12 hr. Upon increasing day length upto 16 hours and increasing luminous intensity can grow the vegetation and stevioside stages of this plant [17], [18].

Standards and criteria for

Congruous season for collection

The harvesting of stevia is depending on the land properties, variety and season. The majority of crops can be collected after the four months of plantation and next crop can be collected once after each 3 months. The best crop collecting time is mid-September to late September when plants are

5070 cm tall. Short days induce flowering. The harvesting of stevia is done just before the flowering because we have got maximum steviol glycoside form the leaves [19].

Adiquate Method of Collection

The leaves are collected in to the baskets for stopping the machine-driven destruction or contamination. The harvesting of stevia is done by the cutting of branches with shear before removing the leaves. the ideas of the stems are often cessed off and added to chop because the maximum amount of stevioside exists in it due to the presence of leaves. on the typical, three marketable crops are often got annually. The best way of cut the pants leaving approx. 9-10 cm stem slice form the bottommost. this may expedite fresh flushes to appear, which may be reaped because of the next harvest. For local usage, fresh leaves used as tea and also in combination with mint leaves [20].

Drying Methods:

The medicinal potency, quality, and commercial value of the stevia reduce at high temperatures [21]. The herbs are dried immediately after the harvesting dextrously on a glass sheet or net. These freshly harvested plants are often hung up in a wrong way and dried in shade by using simple drying racks, which are adjusted inside the transparent poly house or transparent glass roofing. It may also be dried by passing dry air just above temperature. In large scale productions, sometime drying wagon, a kiln can be employed or it may be done by natural process. These kinds of process generally dried stevia within 24 to 48 hours at 40 to 50ºC. There should be proper air circulation and temperature should not be excessive. In moderately warm fall day, stevia is often quickly dried within the full sun. sun drying method is preferrable method over the home dehydrator. After substantial drying, the leaves are barished of the stems/twigs, packed and stored during a cool and dry place [20].

International standards for plant materials

There are some international standards which have been followed by Stevia as a dietary supplement and medicinal plant material. The significant chemical features of dried stevia on the base of GRAS notice by the FDA are indicated in **Table 1**.

AOAC= Association of Official Analytical Chemists; BAM= Bacteriological annual manual;
CFU= Colony-forming unit; ICP=Inductively coupled plasma;

One of the most parameters is the measurement of sweetening properties by measuring the steviol and steviosides and rebaudiana, which delimit four different qualities of stevia through the ultraviolet-visible (UV-Vis) spectrophotometry. Issue in 2018 included some important amendments and questioned by the world enterprises, regarding the mintage of adulteration. The four quality categories for the stevia threads or powder were reduced the three, determined by the glycoside present within the stevia powder.

Criteria according to the food and drug administration (FDA)

Based on the GRAS recognition rules, stevia is permitted by the FDA as a natural sweetener and medicinal use without limitation in culinary purposes; hence, the manufactured goods must follow the below standards.

- Maximum amount of total Ash value should be less than 1% and hence the amount of soluble ash less than 1%.
- The acceptable limit of heavy metals such as Pb, As, Hg must below 1%.
- The leave of stevia collected fresh and dried it at 40-50 ºC FOR 12-48 Hours and also dried in sunlight for 12 hours to maintain the therapeutic properties.

Based on glycosides contented, Sun fruits limited Pune, India, has newly advanced three diversities suitable for different climatic conditions. Description as follows:

optimum management practices.

- SRV–123: contains glycosides content of approx 9–12%. a complete of five cutting per annum are often taken under optimum management practices.
- SRV–512: contains glycosides content approx. 9-12%. This variety is best suited for North Indian conditions and 4 cutting are often taken per annum.
- SRV–128: This sort of stevia is best suitable for everywhere country and contains glycosides content of 21%. It can provide four cuttings per annum with better yield performance [22].

Microbial Pollutants:

Stevia leaves generally blooms on the soil surface containing organic fertilizers and compost hence it is the source of microorganisms. Aerobic spore bacteria like mold, yeast and salmonella spp are usually presents on the microbial flora of these stevia leaves. The sterilization method for micro-propagation is performing with 70% ethanol and 1-3% NaOCl. it isn't suggested because it stimuli

on taste, colour, and odour of the products as found within the literature, chemical sterilization should be more useful [23], [24].

Adulterants

The mixing of stevia with materials like Sodium cyclamate and sodium saccharin are occasionally observed for decreasing the value of stevia. White crystal of stevia has been mixed with the sodium cyclamate to extend the mass of products. Sometimes the sodium saccharin is unfairly mixed with natural stevia. it's reported that the adulterants are loaded with glucose which yielded on incineration to extend ash. Another adulterant which mostly utilized in the stevia is Maltodextrin as a bulking agent [25].

Raman spectroscopy was used to detection of the adulterants within the stevia. This had been capable to identify the sodium cyclamate contents as low as 5% (w/w) during the quantification of stevia-sodium cyclamate mixture. The results indicate that the Raman Spectroscopy can successfully detects the adulterants which not produces any therapeutics effects and even injurious, from the stevia and food [26].

Method of Purity Determination

The remaining a part of residue obtained by the extraction with n-butyl alcohol and water extract of leaf material were processed for the partial purification. The obtained extracts were dissolved in methanol by gentle heating and cooled. Crystal formed after cooling were filtered and washed with methanol. The results of TLC showed that the steviosides and rebaudioside- A were the major and minor compounds among all the steviol glycosides. HPLC is effective one process than the other detection methods [27]. Some amount of residue containing sweet steviol glycoside was dissolved in methanol and mixed with chromatography grade colloid (60-120 mesh, 20 g). the mixture was completely mixed with the help of spatula and methanol. After the evaporation of methanol, the sample was completely dried in vacuum desiccator. The obtained mixture was eluted with chloroform: methanol (95:5 to 85:15) after loading on clean and dry glass column having 60- 120 mesh size. About 50 ml sample were collected after complete distillation of solvent and dissolved in methanol. The fractions were analysed on pre- coated colloidal TLC plates with chloroform: methanol: water (60: 30: 10). Iodine and vitriol (10%) were used for the visualization of spots. All the fractions having same compounds appeared as a single spot. These fractions were concentrated and processed for vacuum drying in combination. The precipitated crystals of steviosided obtained after refluxation and cooling were separated by filtration [28]. On the other hand, the mother liquor was also heated, concentrated and dried in vacuum. Further it was treated with ethanol and water (9:1) [29]. The received product was heated again at a coffee temperature for 45 min and allowed to chill. Precipitate was filtered and dried. The same process was applied again which helps to obtained rebaudioside-A crystals. Authenticity and purity of the isolated compounds were assured by running on TLC plates along with reference compounds. Finally, the melting points of both compounds (steviosides-198º; rebaudioside-A-243ºC) were recorded and matched with the literature values.

CHEMICAL CONSTITUEN

Stevia contains steviosides, rebaudiosides (A, B, C,) and steviol. It also consists dulcoside A. The chemical structure of the chemical constituents shown in Fig. 1, 2,3 and Fig. 4.

PHARMACOLOGICAL ACTIVITY

Anti-diabetic Activity:

Assial A. A *et al.,* (2019) performed the anti-hyperglycemic action of aqueous extract of leaves of *Stevia rebaudiana* by using Macaulay methods in rats. It was observed that the aqueous extract of leaf had significant anti-hyperglycemic activity. The extract decreased the TC and TG level and also enhanced the HDL level in diabetic treated rats. The final result was increased in insulin secretion. Metformin was used as a reference drug [30].

Ahmad U. *et al.,* (2018) performed the anti-hyperglycemic activity of aqueous and ethanolic extract of *Stevia rebaudiana* leaves by using GOD PAP Enzymatic Colorimetric Test Method in the albino rats. It was originate that aqueous extract of leaf of *Stevia rebaudiana* give significant anti-diabetic activity because the aqueous extract having potency to increase the insulin level that act as anti-diabetic activity [31].

Aghajanyan A. *et al.,* (2017) tested the anti-hyperglycemic action of aqueous extract of leaf of *Stevia rebaudiana* by using hydroponics methods in the rabbit. It was found that aqueous extract of leaf of *Stevia rebaudiana* showed significant anti-hyperglycemic activity for sugars and fatty acids in the blood, liver with aqueous extract of *Stevia rebaudiana* [32].

Hepatoprotective Activity

Erika *et al., (*2019)performed the liver damage (cirrhosis) activity of Aqueous Extract of *Stevia rebaudiana* leaf by using analyzed western blotting, qRT-PCR methods in Male Wistar rats. It was found an aqueous extract of *Stevia rebaudiana* leaf showed a significant effect in liver damage activity [33].

Antioxidant activity:

Mutmainah*et al.,* (2019) were used DPPH radical methods for the antioxidant activity of aqueous extract of *Stevia rebaudiana* leaf. Sample was analysed by spectrophotometer and it had been found that the aqueous extract had potent antioxidant activity due to the presence of steviosides [34].

Marisa R. *et al.,* (2018) performed the antioxidant activity of aqueous extract of leaf of *Stevia rebaudiana* by using DPPH radical method, the sample was analyzed by spectrophotometer. it had been found that the found aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity, because N fertilization provide an improvement within the chemical composition and bioactive potential of stevia leaves, the result was expressed certain N2 EC50 =30.06±4.33 mg/ml. and N1 EC50 = 31.21±1.63 mg/ml [35].

Ana G. *et al*., (2018)performed the Antioxidant Activity of Aqueous Extract of leaf of *Stevia rebaudiana* by using Ultrasound-Assisted Extraction (UAE) methods, the sample was analyzed by lspectrophotometer. it was 603.24±3.5) µmol TE/g dw) that mean aqueous Extract of *Stevia rebaudiana* leaf due steviosides as active constituent that showed significant antioxidant activity. Stevia leaf constitutes a possible source of polyphenolic compound, with antioxidant activity [36].

Raut. D *et al.,* (2017) performed the Antioxidant activity of methanolic extracts Extract of leaf of *Stevia rebaudiana* by using DPPH radical methods, the sample was analyzed by spectrophotometer. it had been found that the found aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity, the result was expressed IC50 value of methanolic extract of stevia and ascorbic acid were found to be 32.765 µg/ml and 6.474 µg/ml. this antioxidant activity due to methanolic extract of *Stevia rebaudiana* was found antioxidants molecules like Delphinidin, rosmarinic acid, vitamin C is employed as reference drug [37].

Javed. R *et al.,* (2016) performed the Antioxidant Activity of Aqueous Extract of leaf of *Stevia rebaudiana* by using DPPH radical methods, the sample was analyzed by spectrophotometer. it had been found that the found aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity, because of ZnO nanoparticle implicated oxidative stress by release the metal ions or radical in MS medium the result optimized dose were found to be 1 mg L-1 [38].

Juana M *et al.,* (2015)performed the Antioxidant Action of Aqueous Extract of leaf of *Stevia rebaudiana* by using TEAC, ORAC, and DPPH free radical scavenging assay methods. It was found that the aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity due to the significance of the rise in the bioaccessibility of bioactive compounds in blood. It was found with combination S. rebaudiana at 1.25% (24.1±0.2 mm TE) and 2.5% (35.5±0.6 mm TE) [39].

Gawal-Beben. K *et al.,* (2015) performed the Antioxidant activity of aqueous, ethanolic (E) and glycol-aqueous (GA) Extract of leaf of *Stevia rebaudiana* by using DPPH radical scavenging assay methods, it was aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity. thanks to the many cytotoxicity of E and GA extracts also as their fibroblast irritating the acceptable dose of extract especially food or cosmetic products for showing antioxidant activity [40].

J. C. Ruiz-Ruiz *et al.,* (2015) performed the Antioxidant Activity of Aqueous Extract of the leaf of *Stevia rebaudiana* by using DPPH radical scavenging assay methods; it had been found that the aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity. The IC50 value= 335.94 µg/ml. this activity because the ability to scale back the sugar consumption was emphasised by acting enzymatic assays using α-amylase and α-glucosidase [41].

B. Gopal Krishnan *et al.,* (2006)Performed the Antioxidant activity of ethanolic extract of leaf of *Stevia rebaudiana* by using DPPH free radical scavenging assay methods, It was showed IC_{50} value was found to be 140 µg compared with the IC_{50} 76 µg value BHT, It was found of *Stevia rebaudiana* leaf showed significant antioxidant activity [42].

Anti-Microbial Activity:

Darshana Raut *et al., (*2017)Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of the leaf of *Stevia rebaudiana* by using Agar-dilution methods in albino rats, It was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, Methanol extract shows the zone of inhibition ranged from (18-24 mm) as compared to aqueous extract minimum bactericidal concentration (MBC) range from (10-20 mg/ml) [36].

Mali A B. *et al.,* (2015) Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of the leaf of *Stevia rebaudiana* by using Disc diffusion methods in albino rats, It was found that solvent Extract

of *Stevia rebaudiana* leaf showed significant antibacterial activity, the zone of inhibition for B. subtilus showed maximum zone of inhibition = 18.6 mm and the minimum is 13.8 mm, against ethanolic extract, E Coli showed maximum zone of inhibition 11.8 mm and minimum zone of inhibition= 8 mm extract by soxhlet method, and extract from column showed very less zone of inhibition against E. aerogenes = 10 mm and E coli= 7mm [43].

Maryam Mohd.-Sichani*et al., (*2012)Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of leaf of *Stevia rebaudiana* by using Agar-well diffusion methods in albino rats, It was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity [44].

Francois N M. *et al.,* (2011) Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of the leaf of *Stevia rebaudiana* by using Disc diffusion methods in albino rats, it had been found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, the extract with EO, WE, MWE the Minimum inhibition concentration (MIC) was found to be in acetone extract: S. aureus = >1000 (EO), >700 (WE), >500 (ETWE), >500 (MWE) same as for Bacillus subtulis, E. coli or Candida albicans except for *Aspergillus niger*>1000 (EO), NIL for (WE), >700 (ETWE), >700 (MWE) and for P. aeruginosa NIL (EO) >700 (WE), >500 (ETWE), >500 (MWE) [45].

S Jayaraman *et al.,* [46] Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of leaf of *Stevia rebaudiana* by using Agar-well diffusion methods in albino rats, It was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, the zone of inhibition was found to be in acetone extract: S. aureus = 19 mm., Bacillus subtulis = 18 mm and Ethyl acetate extract very effective against Vibrio cholera = 18 mm.

Ghosh S. *et al.,* (2008)Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of leaf of *Stevia rebaudiana* by using Plate dilution methods for MIC and Diffusion method for Zone of Inhibition in albeno rats, it was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, the zone of inhibition was found to be in Petroleum ether extract: have highest A_bI against S. aureus, E. faecalisP. aeruginosa IZD as 16.3 mm, 13 mm, 11 mm and ethanolic, water and acetone extract the IZD= 11 mm, 10.6 mm, 10.3 mm [47].

Cosmetic Formulation:

K Das *et al*., (2009)Performed the skin moisturizing activity of aqueous leaf extract of *Stevia rebaudiana* by using Physiological Measurement in comparison with a control placebo gel, it was found that aqueous extract of *Stevia rebaudiana* leaf showed significant moisturizing activity [48].

Anti-Fungal Activity:

Shukla S. *et al.,* (2013) Performed anti-fungal activity of ethanolic and aqueous Extract of *Stevia rebaudiana* by using disc diffusion method, in albino rats, it had been found that aqueous extract of *Stevia rebaudiana* leaf showed significantly the antifungal activity due stevia inhibit the fungal growth that observed by radical growth inhibition resistant to B. cinerea (64.2 and 67.5 %), whereas Minimum Inhibition concentration found to be 1-3 mg/ml [49].

Anti-Tumor Activity:

Antitumor activity of methanolic, ethanolic and aqueous extract of leaves of *Stevia rebaudiana* was tested by applying MTT Assay methods by Jayaraman**.** After the study, it was found that aqueous extract of *Stevia rebaudiana* demonstrated cytotoxic effect HE_{p2} cells [46].

Dental Caries oral hygiene

Sharma S. *et al.,* (2014)Performed the oral hygiene activity of polyherbal extract of *Stevia rebaudiana* leaf by using of serial micro-dilution method on the rats, it was found that polyherbal extract of *Stevia rebaudiana* leaf Showed significant anti-oral hygiene activity the extract showed inhibitory activity with increase the concentration of stevia, FI formulation (500mg/ml) MBC of polyherbal methanolic extract shows zone inhibition diameter ranging from (17.6-26.1 mm), for F2 formulation (250 mg/ml) was shows zone inhibition diameter ranging from (9.0 -12.8 mm) due to this F1 formulation shows more effective than other formulation for dental hygiene [50].

Packaging and Storage:

Factor that shows decomposition or decrease the standard are as follows: Humidity of the products and relative air humidity, temperature of the around direct sunlight, oxygen, and superiority of Packages, it's clear that the lower the temperature and humidity, the upper the standard. Once dried, whole stevia leaves are often stored for 12 months within the air-tight containers or plastic bags to increase their self-life, lookout of humidity. Once the leaves are dry you'll crush them into a fine powder. Use a mesh screen or grind them during a kitchen by coffee mill. Dry leaves are saved in plastic-lined wooden boxes, wrapped, strapped, and labelled for additional processing. After powdering it's to be packed and labelled properly [51].

Table 1. Chemical features of dried stevia on the base of GRAS notice by the FDA

Parameters	Specification	Method of analysis Used Cargill
IDENTITY		
Assay (steviol glycosides)	NLT 95%	STV-002-06
Appearances	Loose of powder, crystals, white to off-white	STV-003-01
PURITY		
Ash	NLT 1.0%	AOAC945.46
Loss on drying	NMT 6.0%	STV-006-02
Residual Solvents	NMT 0.02 Methanol NMT 0.5 Ethanol	STV-009-01
HEAVY METALS		
Arsenic	NMT 1 mg/kg	USP 730 ICP-MS
Cadmium	NMT 1 mg/kg	USP 730 ICP-MS
Lead	NMT 1 mg/kg	USP 730 ICP-MS
Mercury	NMT 1 mg/kg	USP 730 ICP-MS
MICROORGANISMS		
Aerobic plate count	LT 1000 CFU/g	AOAC 966.23
Yeast	NMT 50 CFU/g	FDA BAM, 7th edition
Mold	NMT 50 CFU/g	FDA BAM, 7th edition
Salmonella spp.	Negative/25 g	AOAC-R1 100201

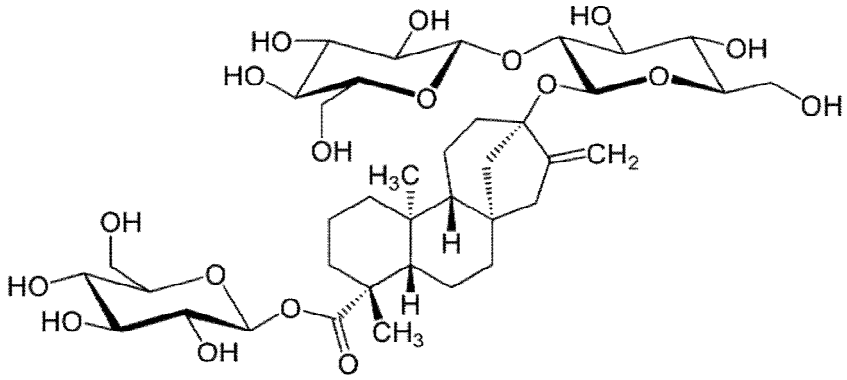


Fig. 1. Steviosides

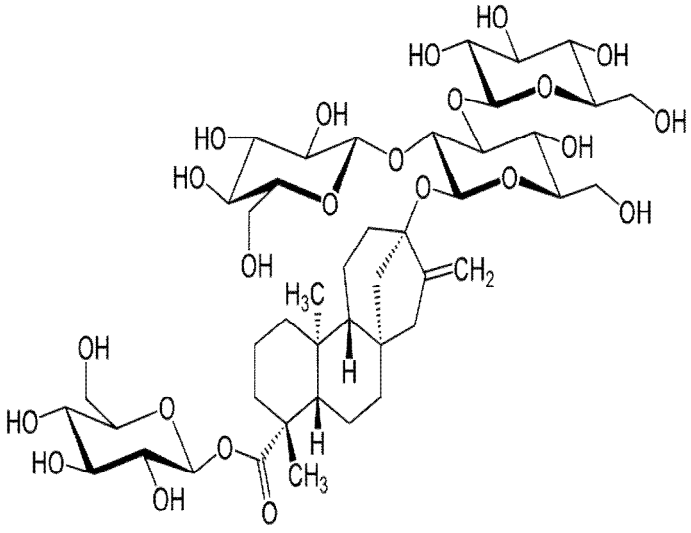


Fig. 2. Rebaudiosides A

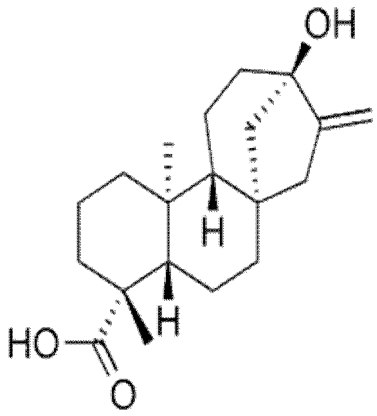
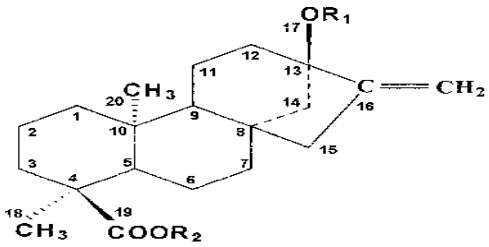


Fig. 3. Steviol



Compound		R_1	R_2
Stevioside	(1)	-glc-^{2}-glc	-glc
Steviolbioside	(2)	-glc-^{2}-glc	-H
Rebaudioside A	(3)	glc-^{3}-glc-^{2}-glc	-glc
Rebaudioside B	(4)	glc-^{3}-glc-^{2}-glc	-H
Rebaudioside C	(5)	glc-^{3}-glc-^{2}-rha	-glc
Dulcoside A	(6)	-glc-^{2}-rha	-glc
Steviol	(7)	-H	-H

Fig. 4. Basic Chemical Structure of *Stevia rebaudiana* constituents.

CONCLUSION

Stevia rebaudiana is of essential remedial food plants developing usually in Paraguay, Brazil, and also cultivated for his or her dietary resolves and financial significance. The plant *Stevia rebaudiana* is usually spread in Paraguay, Central America, Brazil, China, Korea, and Thailand, Stevia cultivates in well-drained fertile soil having more organic matter. At acid to neutral (pH 6-7) soil with proper supply of moisture effective for growth, but not waterlogged fields. Now a day's stevia is cultivated in Paraguay Brazil and a couple of countries with oil refinement. Stevia is the best natural sugar and having medicinal potency and by diabetic patients, it's used as sugar. Restriction of manufacture and high demand for depletion resulted within the most potent and worth of stevia. The current literature about the pharmacological action of stevia makes it the thing of regular adulteration and frauds, and the object of varied phytochemical and biotechnological investigates. Furthermore, the authorization of the origin and excellence of stevia as a medicinal food led to the frequent usages of chemical and molecular techniques. Determining of chemical composition of stevia is another effort for preventing the stevia adulteration. The TLC, Colum chromatography, HPLC analytical procedures are sensitive, reproducible, and permit obtaining a sufficient amount of stevia component (Stevioside, Steviol, Rebaudioside-A) for further analytical assessments. Additionally, APCI-MS techniques, FT-NIR spectroscopy analysis, and UV-Vis spectrometry are mainly used for qualitative and quantitative chemical analysis. The humidity, temperature of direct sunlight and quality of packages are the ultimate parameters that impact on the standard of the product.

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Advances in Bioresearch

REVIEW ARTICLE

Phytochemicals and Medicinal Properties of *Stevia rebaudiana*: A Review

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ABSTRACT
Stevia is used as an ayurvedic diet supplement because it is the most potential in Brazil, China agronomic budget. The dry leaves of Stevia rebaudiana (Asteraceae) are dealing out to produce Steviol and Stevioside as an identified natural sugar that is used in pharmaceutics, cosmetics, and food productions. Newly, reports about the therapeutic effect of stevia rise its significance in the globes. The yearly harvesting yield of stevia is valued approx 600 tons yearly (China harvests 75% of total) globally, and also stevia is reflected to be the most effective and valuable as sugar substitute for diabetic patients in the world; due to this there are chance of artificial production or defraud. Due to this maintenance of the quality of stevia and requirements of a certification by following GRAS, ISO, or the Food and Drug Administration (FDA) criteria and standards. In this review, the current (or sometimes less documented) information on Pharmacognosy, pharmacology, and ordinary approaches for quality estimate of stevia, as a therapeutic nutrition herbal tea, from field cultivation to market are reviewed.
Keywords*: Stevia rebaudiana, Asteraceae, phytochemistry, stevia, standardization, steviosides, rebaudioside- A.*

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INTRODUCTION

Stevia rebaudiana is an herbaceous, semi-bushy, perennial shrub (Asteraceae family) and it ia also well acknowledged as sweet leaf, honey leaf, candy leaf, sweet weed, or sweet herbs Natural Sweetener in the world [1]. Gaurani Indians widely recycled this shrub since 1500 years in South America (Paraguay, Brazil), who called it ka'ahe'e ("sweet herb") [2], the genus was termed for Spanish botanist and physician PetrusJacobus Stevus, a professor of botany at the University of Valencia [3]. Dr. Moises Santiago Bertoni is first coin the word stevia and discovered this shrub in 1888 at Paraguay. Paraguayan chemist Dr. Rebaudi in 1905, the plant was systematically called as S. rebaudiana. In 12/2008, the FDA provided a "no objection" authorization for GRAS status to Truvia which is industrialized by Cargill and Coca-Cola Company [4], it means that the pure stevia as plant or shrub is not safe for use in the dietary supplement but a highly purified product of this plantsis used as a safe dietary supplement [5]. In 2017, great pureness stevia glycosides are deliberated non-toxic and allowable as an ingredient in food products sold within the United State [6]. The outstanding features of the stem of stevia sweet within the taste which don't contain active compounds usually aren't collected. The leave of the stevia with carbohydrate-based compounds that's 2-3 hundred times sweeter than sugar. It is found that stevia contains alkaloid such as steviosides which shows insulin-tropic properties in pancreatic beta cells. It reduces the blood sugar level by increasing the insulin secretion. It is used as substitute sweeteners for sugars [7]. Currently, average yields reported fall between 2494-3628 kg per acre with the plant production within the lower range. the wealth for conservative manufacture is about $1.254 per kg and herb production is $0.75 per pound in New Bern, NC [8].

CLASSIFICATION

Traditional Usage:

Stevia has been used in dietary supplements as a non-caloric Sweetener [9]. Beside through sugariness, an unpleasant taste is also identified in humans [10]herbal tea, Natural sugar, and Ayurvedic health system as Anti-diabetic, Anti-obesity, and Anti-cholesterol. Many pasts on the usage of S. rebaudiana are given by the antediluvian Ayurvedic system of medicine [11]. S. rebaudiana leaves has been endorsed as a cure in contradiction of several prolonged and non-chronic diseases like renal disease, diabetes, cancer, obesity, inflammatory bowel disease, cardiovascular disease, and dental caries.

Common Name of *Stevia rebaudiana*:

Stevia, candy leaf sweet leaf of Paraguay, sweet-herb, honey yerba, honey leaf, yaawaan,

Vernacular Names

Hindi: meethipatti
English: Sweetleaf, Honey leaf, Sweet herb
French: Stévia or Stévie
Marathi: MadhuParani
Sanskrit: MadhuPatra
Tamil: SeeniTulsi
Telugu: MadhuPatri

Taxonomical classification:

Kingdom: Angiospermae
Class: Dicotyledons
Group: Monochlamydae
Order: Asterales
Family: Asteraceae
Subfamily: Asteroideae
Tribe: Eupatorieae
Genus: Stevia
Species: rebaudiana

Plant description

Macroscopically Characteristics:

Stevia iscultivating up to 1m tall and has leaves 2-3 cm long. Macroscopic character expressed by Fig 1

Leaves –Sessile Green in color.
Odour- Odourless.
Taste- sweetish
Size- 5 cm in length and 3 cm in width
Shape- ovate
Extra features- leaves acuminate petiolate, faces are glabrous
Flower- white, throats funnel form lobes [12].

Climate and Land required for Cultivation:

S. rebaudiana is that the best remedial food plant, as a source of stevia, which has highest exporting status in china approx. 75% of the export of stevia from china that's by china is the biggest exporter in the world and Paraguay, Central America, Korea, and Thailand. The cultivated land area of stevia in all over the world is 32000 hectares. Notwithstanding china is the best region for development, Central America and Brazil are the appropriate areas for the cultivation of stevia. Thailand Korea and India are the most suitable country for the cultivation of stevia. In Stevia may be a perennial herb natural to between 22°-24° south and 53°-56° west in Paraguay and Brazil [13], [14]. stevia developed at higher latitudes even have a better proportion of sugary glycosides [15]. Nourishment and climate show vital roles in the expansion and secondary metabolites of stevia plants [16]. At low temperature (below 20 ºC) and day size is smaller amount then 12 hr. Upon increasing day length upto 16 hours and increasing luminous intensity can grow the vegetation and stevioside stages of this plant [17], [18].

Standards and criteria for

Congruous season for collection

The harvesting of stevia is depending on the land properties, variety and season. The majority of crops can be collected after the four months of plantation and next crop can be collected once after each 3 months. The best crop collecting time is mid-September to late September when plants are

5070 cm tall. Short days induce flowering. The harvesting of stevia is done just before the flowering because we have got maximum steviol glycoside form the leaves [19].

Adiquate Method of Collection

The leaves are collected in to the baskets for stopping the machine-driven destruction or contamination. The harvesting of stevia is done by the cutting of branches with shear before removing the leaves. the ideas of the stems are often cessed off and added to chop because the maximum amount of stevioside exists in it due to the presence of leaves. on the typical, three marketable crops are often got annually. The best way of cut the pants leaving approx. 9-10 cm stem slice form the bottommost. this may expedite fresh flushes to appear, which may be reaped because of the next harvest. For local usage, fresh leaves used as tea and also in combination with mint leaves [20].

Drying Methods:

The medicinal potency, quality, and commercial value of the stevia reduce at high temperatures [21]. The herbs are dried immediately after the harvesting dextrously on a glass sheet or net. These freshly harvested plants are often hung up in a wrong way and dried in shade by using simple drying racks, which are adjusted inside the transparent poly house or transparent glass roofing. It may also be dried by passing dry air just above temperature. In large scale productions, sometime drying wagon, a kiln can be employed or it may be done by natural process. These kinds of process generally dried stevia within 24 to 48 hours at 40 to 50ºC. There should be proper air circulation and temperature should not be excessive. In moderately warm fall day, stevia is often quickly dried within the full sun. sun drying method is preferrable method over the home dehydrator. After substantial drying, the leaves are barished of the stems/twigs, packed and stored during a cool and dry place [20].

International standards for plant materials

There are some international standards which have been followed by Stevia as a dietary supplement and medicinal plant material. The significant chemical features of dried stevia on the base of GRAS notice by the FDA are indicated in **Table 1**.

AOAC= Association of Official Analytical Chemists; BAM= Bacteriological annual manual;
CFU= Colony-forming unit; ICP=Inductively coupled plasma;

One of the most parameters is the measurement of sweetening properties by measuring the steviol and steviosides and rebaudiana, which delimit four different qualities of stevia through the ultraviolet-visible (UV-Vis) spectrophotometry. Issue in 2018 included some important amendments and questioned by the world enterprises, regarding the mintage of adulteration. The four quality categories for the stevia threads or powder were reduced the three, determined by the glycoside present within the stevia powder.

Criteria according to the food and drug administration (FDA)

Based on the GRAS recognition rules, stevia is permitted by the FDA as a natural sweetener and medicinal use without limitation in culinary purposes; hence, the manufactured goods must follow the below standards.

- Maximum amount of total Ash value should be less than 1% and hence the amount of soluble ash less than 1%.
- The acceptable limit of heavy metals such as Pb, As, Hg must below 1%.
- The leave of stevia collected fresh and dried it at 40-50 ºC FOR 12-48 Hours and also dried in sunlight for 12 hours to maintain the therapeutic properties.

Based on glycosides contented, Sun fruits limited Pune, India, has newly advanced three diversities suitable for different climatic conditions. Description as follows:

optimum management practices.

- SRV–123: contains glycosides content of approx 9–12%. a complete of five cutting per annum are often taken under optimum management practices.
- SRV–512: contains glycosides content approx. 9-12%. This variety is best suited for North Indian conditions and 4 cutting are often taken per annum.
- SRV–128: This sort of stevia is best suitable for everywhere country and contains glycosides content of 21%. It can provide four cuttings per annum with better yield performance [22].

Microbial Pollutants:

Stevia leaves generally blooms on the soil surface containing organic fertilizers and compost hence it is the source of microorganisms. Aerobic spore bacteria like mold, yeast and salmonella spp are usually presents on the microbial flora of these stevia leaves. The sterilization method for micro-propagation is performing with 70% ethanol and 1-3% NaOCl. it isn't suggested because it stimuli

on taste, colour, and odour of the products as found within the literature, chemical sterilization should be more useful [23], [24].

Adulterants

The mixing of stevia with materials like Sodium cyclamate and sodium saccharin are occasionally observed for decreasing the value of stevia. White crystal of stevia has been mixed with the sodium cyclamate to extend the mass of products. Sometimes the sodium saccharin is unfairly mixed with natural stevia. it's reported that the adulterants are loaded with glucose which yielded on incineration to extend ash. Another adulterant which mostly utilized in the stevia is Maltodextrin as a bulking agent [25].

Raman spectroscopy was used to detection of the adulterants within the stevia. This had been capable to identify the sodium cyclamate contents as low as 5% (w/w) during the quantification of stevia-sodium cyclamate mixture. The results indicate that the Raman Spectroscopy can successfully detects the adulterants which not produces any therapeutics effects and even injurious, from the stevia and food [26].

Method of Purity Determination

The remaining a part of residue obtained by the extraction with n-butyl alcohol and water extract of leaf material were processed for the partial purification. The obtained extracts were dissolved in methanol by gentle heating and cooled. Crystal formed after cooling were filtered and washed with methanol. The results of TLC showed that the steviosides and rebaudioside- A were the major and minor compounds among all the steviol glycosides. HPLC is effective one process than the other detection methods [27]. Some amount of residue containing sweet steviol glycoside was dissolved in methanol and mixed with chromatography grade colloid (60-120 mesh, 20 g). the mixture was completely mixed with the help of spatula and methanol. After the evaporation of methanol, the sample was completely dried in vacuum desiccator. The obtained mixture was eluted with chloroform: methanol (95:5 to 85:15) after loading on clean and dry glass column having 60- 120 mesh size. About 50 ml sample were collected after complete distillation of solvent and dissolved in methanol. The fractions were analysed on pre- coated colloidal TLC plates with chloroform: methanol: water (60: 30: 10). Iodine and vitriol (10%) were used for the visualization of spots. All the fractions having same compounds appeared as a single spot. These fractions were concentrated and processed for vacuum drying in combination. The precipitated crystals of steviosided obtained after refluxation and cooling were separated by filtration [28]. On the other hand, the mother liquor was also heated, concentrated and dried in vacuum. Further it was treated with ethanol and water (9:1) [29]. The received product was heated again at a coffee temperature for 45 min and allowed to chill. Precipitate was filtered and dried. The same process was applied again which helps to obtained rebaudioside-A crystals. Authenticity and purity of the isolated compounds were assured by running on TLC plates along with reference compounds. Finally, the melting points of both compounds (steviosides-198º; rebaudioside-A-243ºC) were recorded and matched with the literature values.

CHEMICAL CONSTITUEN

Stevia contains steviosides, rebaudiosides (A, B, C,) and steviol. It also consists dulcoside A. The chemical structure of the chemical constituents shown in Fig. 1, 2,3 and Fig. 4.

PHARMACOLOGICAL ACTIVITY

Anti-diabetic Activity:

Assial A. A *et al.,* (2019) performed the anti-hyperglycemic action of aqueous extract of leaves of *Stevia rebaudiana* by using Macaulay methods in rats. It was observed that the aqueous extract of leaf had significant anti-hyperglycemic activity. The extract decreased the TC and TG level and also enhanced the HDL level in diabetic treated rats. The final result was increased in insulin secretion. Metformin was used as a reference drug [30].

Ahmad U. *et al.,* (2018) performed the anti-hyperglycemic activity of aqueous and ethanolic extract of *Stevia rebaudiana* leaves by using GOD PAP Enzymatic Colorimetric Test Method in the albino rats. It was originate that aqueous extract of leaf of *Stevia rebaudiana* give significant anti-diabetic activity because the aqueous extract having potency to increase the insulin level that act as anti-diabetic activity [31].

Aghajanyan A. *et al.,* (2017) tested the anti-hyperglycemic action of aqueous extract of leaf of *Stevia rebaudiana* by using hydroponics methods in the rabbit. It was found that aqueous extract of leaf of *Stevia rebaudiana* showed significant anti-hyperglycemic activity for sugars and fatty acids in the blood, liver with aqueous extract of *Stevia rebaudiana* [32].

Hepatoprotective Activity

Erika *et al., (*2019)performed the liver damage (cirrhosis) activity of Aqueous Extract of *Stevia rebaudiana* leaf by using analyzed western blotting, qRT-PCR methods in Male Wistar rats. It was found an aqueous extract of *Stevia rebaudiana* leaf showed a significant effect in liver damage activity [33].

Antioxidant activity:

Mutmainah*et al.,* (2019) were used DPPH radical methods for the antioxidant activity of aqueous extract of *Stevia rebaudiana* leaf. Sample was analysed by spectrophotometer and it had been found that the aqueous extract had potent antioxidant activity due to the presence of steviosides [34].

Marisa R. *et al.,* (2018) performed the antioxidant activity of aqueous extract of leaf of *Stevia rebaudiana* by using DPPH radical method, the sample was analyzed by spectrophotometer. it had been found that the found aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity, because N fertilization provide an improvement within the chemical composition and bioactive potential of stevia leaves, the result was expressed certain N2 EC50 =30.06±4.33 mg/ml. and N1 EC50 = 31.21±1.63 mg/ml [35].

Ana G. *et al*., (2018)performed the Antioxidant Activity of Aqueous Extract of leaf of *Stevia rebaudiana* by using Ultrasound-Assisted Extraction (UAE) methods, the sample was analyzed by lspectrophotometer. it was 603.24±3.5) µmol TE/g dw) that mean aqueous Extract of *Stevia rebaudiana* leaf due steviosides as active constituent that showed significant antioxidant activity. Stevia leaf constitutes a possible source of polyphenolic compound, with antioxidant activity [36].

Raut. D *et al.,* (2017) performed the Antioxidant activity of methanolic extracts Extract of leaf of *Stevia rebaudiana* by using DPPH radical methods, the sample was analyzed by spectrophotometer. it had been found that the found aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity, the result was expressed IC50 value of methanolic extract of stevia and ascorbic acid were found to be 32.765 µg/ml and 6.474 µg/ml. this antioxidant activity due to methanolic extract of *Stevia rebaudiana* was found antioxidants molecules like Delphinidin, rosmarinic acid, vitamin C is employed as reference drug [37].

Javed. R *et al.,* (2016) performed the Antioxidant Activity of Aqueous Extract of leaf of *Stevia rebaudiana* by using DPPH radical methods, the sample was analyzed by spectrophotometer. it had been found that the found aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity, because of ZnO nanoparticle implicated oxidative stress by release the metal ions or radical in MS medium the result optimized dose were found to be 1 mg L-1 [38].

Juana M *et al.,* (2015)performed the Antioxidant Action of Aqueous Extract of leaf of *Stevia rebaudiana* by using TEAC, ORAC, and DPPH free radical scavenging assay methods. It was found that the aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity due to the significance of the rise in the bioaccessibility of bioactive compounds in blood. It was found with combination S. rebaudiana at 1.25% (24.1±0.2 mm TE) and 2.5% (35.5±0.6 mm TE) [39].

Gawal-Beben. K *et al.,* (2015) performed the Antioxidant activity of aqueous, ethanolic (E) and glycol-aqueous (GA) Extract of leaf of *Stevia rebaudiana* by using DPPH radical scavenging assay methods, it was aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity. thanks to the many cytotoxicity of E and GA extracts also as their fibroblast irritating the acceptable dose of extract especially food or cosmetic products for showing antioxidant activity [40].

J. C. Ruiz-Ruiz *et al.,* (2015) performed the Antioxidant Activity of Aqueous Extract of the leaf of *Stevia rebaudiana* by using DPPH radical scavenging assay methods; it had been found that the aqueous Extract of *Stevia rebaudiana* leaf showed significant antioxidant activity. The IC50 value= 335.94 µg/ml. this activity because the ability to scale back the sugar consumption was emphasised by acting enzymatic assays using α-amylase and α-glucosidase [41].

B. Gopal Krishnan *et al.,* (2006)Performed the Antioxidant activity of ethanolic extract of leaf of *Stevia rebaudiana* by using DPPH free radical scavenging assay methods, It was showed IC_{50} value was found to be 140 µg compared with the IC_{50} 76 µg value BHT, It was found of *Stevia rebaudiana* leaf showed significant antioxidant activity [42].

Anti-Microbial Activity:

Darshana Raut *et al., (*2017)Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of the leaf of *Stevia rebaudiana* by using Agar-dilution methods in albino rats, It was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, Methanol extract shows the zone of inhibition ranged from (18-24 mm) as compared to aqueous extract minimum bactericidal concentration (MBC) range from (10-20 mg/ml) [36].

Mali A B. *et al.,* (2015) Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of the leaf of *Stevia rebaudiana* by using Disc diffusion methods in albino rats, It was found that solvent Extract

of *Stevia rebaudiana* leaf showed significant antibacterial activity, the zone of inhibition for B. subtilus showed maximum zone of inhibition = 18.6 mm and the minimum is 13.8 mm, against ethanolic extract, E Coli showed maximum zone of inhibition 11.8 mm and minimum zone of inhibition= 8 mm extract by soxhlet method, and extract from column showed very less zone of inhibition against E. aerogenes = 10 mm and E coli= 7mm [43].

Maryam Mohd.-Sichani*et al., (*2012)Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of leaf of *Stevia rebaudiana* by using Agar-well diffusion methods in albino rats, It was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity [44].

Francois N M. *et al.,* (2011) Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of the leaf of *Stevia rebaudiana* by using Disc diffusion methods in albino rats, it had been found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, the extract with EO, WE, MWE the Minimum inhibition concentration (MIC) was found to be in acetone extract: S. aureus = >1000 (EO), >700 (WE), >500 (ETWE), >500 (MWE) same as for Bacillus subtulis, E. coli or Candida albicans except for *Aspergillus niger*>1000 (EO), NIL for (WE), >700 (ETWE), >700 (MWE) and for P. aeruginosa NIL (EO) >700 (WE), >500 (ETWE), >500 (MWE) [45].

S Jayaraman *et al.,* [46] Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of leaf of *Stevia rebaudiana* by using Agar-well diffusion methods in albino rats, It was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, the zone of inhibition was found to be in acetone extract: S. aureus = 19 mm., Bacillus subtulis = 18 mm and Ethyl acetate extract very effective against Vibrio cholera = 18 mm.

Ghosh S. *et al.,* (2008)Performed the Antimicrobial activity of solvent (methanol, ethanol) Extract of leaf of *Stevia rebaudiana* by using Plate dilution methods for MIC and Diffusion method for Zone of Inhibition in albeno rats, it was found that solvent Extract of *Stevia rebaudiana* leaf showed significant antibacterial activity, the zone of inhibition was found to be in Petroleum ether extract: have highest A_bI against S. aureus, E. faecalisP. aeruginosa IZD as 16.3 mm, 13 mm, 11 mm and ethanolic, water and acetone extract the IZD= 11 mm, 10.6 mm, 10.3 mm [47].

Cosmetic Formulation:

K Das *et al*., (2009)Performed the skin moisturizing activity of aqueous leaf extract of *Stevia rebaudiana* by using Physiological Measurement in comparison with a control placebo gel, it was found that aqueous extract of *Stevia rebaudiana* leaf showed significant moisturizing activity [48].

Anti-Fungal Activity:

Shukla S. *et al.,* (2013) Performed anti-fungal activity of ethanolic and aqueous Extract of *Stevia rebaudiana* by using disc diffusion method, in albino rats, it had been found that aqueous extract of *Stevia rebaudiana* leaf showed significantly the antifungal activity due stevia inhibit the fungal growth that observed by radical growth inhibition resistant to B. cinerea (64.2 and 67.5 %), whereas Minimum Inhibition concentration found to be 1-3 mg/ml [49].

Anti-Tumor Activity:

Antitumor activity of methanolic, ethanolic and aqueous extract of leaves of *Stevia rebaudiana* was tested by applying MTT Assay methods by Jayaraman**.** After the study, it was found that aqueous extract of *Stevia rebaudiana* demonstrated cytotoxic effect HE_{p2} cells [46].

Dental Caries oral hygiene

Sharma S. *et al.,* (2014)Performed the oral hygiene activity of polyherbal extract of *Stevia rebaudiana* leaf by using of serial micro-dilution method on the rats, it was found that polyherbal extract of *Stevia rebaudiana* leaf Showed significant anti-oral hygiene activity the extract showed inhibitory activity with increase the concentration of stevia, FI formulation (500mg/ml) MBC of polyherbal methanolic extract shows zone inhibition diameter ranging from (17.6-26.1 mm), for F2 formulation (250 mg/ml) was shows zone inhibition diameter ranging from (9.0 -12.8 mm) due to this F1 formulation shows more effective than other formulation for dental hygiene [50].

Packaging and Storage:

Factor that shows decomposition or decrease the standard are as follows: Humidity of the products and relative air humidity, temperature of the around direct sunlight, oxygen, and superiority of Packages, it's clear that the lower the temperature and humidity, the upper the standard. Once dried, whole stevia leaves are often stored for 12 months within the air-tight containers or plastic bags to increase their self-life, lookout of humidity. Once the leaves are dry you'll crush them into a fine powder. Use a mesh screen or grind them during a kitchen by coffee mill. Dry leaves are saved in plastic-lined wooden boxes, wrapped, strapped, and labelled for additional processing. After powdering it's to be packed and labelled properly [51].

Table 1. Chemical features of dried stevia on the base of GRAS notice by the FDA

Parameters	Specification	Method of analysis Used Cargill
IDENTITY		
Assay (steviol glycosides)	NLT 95%	STV-002-06
Appearances	Loose of powder, crystals, white to off-white	STV-003-01
PURITY		
Ash	NLT 1.0%	AOAC945.46
Loss on drying	NMT 6.0%	STV-006-02
Residual Solvents	NMT 0.02 Methanol NMT 0.5 Ethanol	STV-009-01
HEAVY METALS		
Arsenic	NMT 1 mg/kg	USP 730 ICP-MS
Cadmium	NMT 1 mg/kg	USP 730 ICP-MS
Lead	NMT 1 mg/kg	USP 730 ICP-MS
Mercury	NMT 1 mg/kg	USP 730 ICP-MS
MICROORGANISMS		
Aerobic plate count	LT 1000 CFU/g	AOAC 966.23
Yeast	NMT 50 CFU/g	FDA BAM, 7th edition
Mold	NMT 50 CFU/g	FDA BAM, 7th edition
Salmonella spp.	Negative/25 g	AOAC-R1 100201

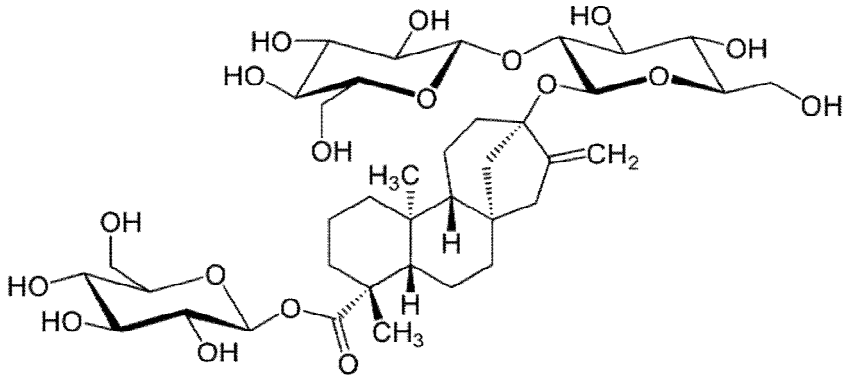


Fig. 1. Steviosides

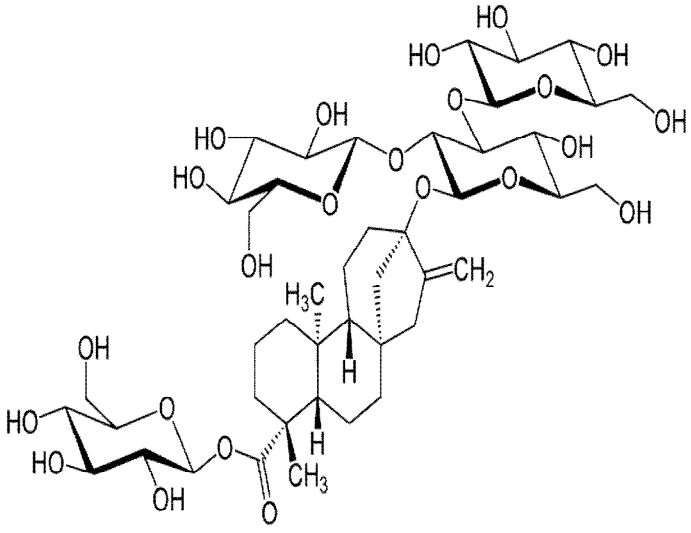


Fig. 2. Rebaudiosides A

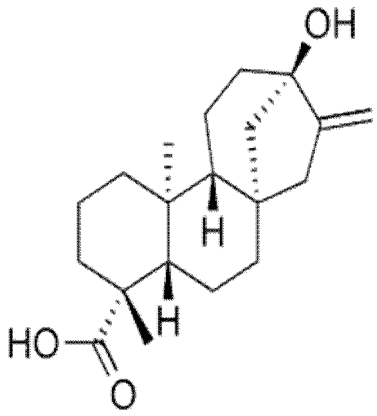
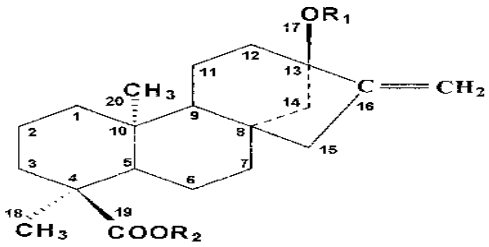


Fig. 3. Steviol



Compound		R_1	R_2
Stevioside	(1)	-glc-^{2}-glc	-glc
Steviolbioside	(2)	-glc-^{2}-glc	-H
Rebaudioside A	(3)	glc-^{3}-glc-^{2}-glc	-glc
Rebaudioside B	(4)	glc-^{3}-glc-^{2}-glc	-H
Rebaudioside C	(5)	glc-^{3}-glc-^{2}-rha	-glc
Dulcoside A	(6)	-glc-^{2}-rha	-glc
Steviol	(7)	-H	-H

Fig. 4. Basic Chemical Structure of *Stevia rebaudiana* constituents.

CONCLUSION

Stevia rebaudiana is of essential remedial food plants developing usually in Paraguay, Brazil, and also cultivated for his or her dietary resolves and financial significance. The plant *Stevia rebaudiana* is usually spread in Paraguay, Central America, Brazil, China, Korea, and Thailand, Stevia cultivates in well-drained fertile soil having more organic matter. At acid to neutral (pH 6-7) soil with proper supply of moisture effective for growth, but not waterlogged fields. Now a day's stevia is cultivated in Paraguay Brazil and a couple of countries with oil refinement. Stevia is the best natural sugar and having medicinal potency and by diabetic patients, it's used as sugar. Restriction of manufacture and high demand for depletion resulted within the most potent and worth of stevia. The current literature about the pharmacological action of stevia makes it the thing of regular adulteration and frauds, and the object of varied phytochemical and biotechnological investigates. Furthermore, the authorization of the origin and excellence of stevia as a medicinal food led to the frequent usages of chemical and molecular techniques. Determining of chemical composition of stevia is another effort for preventing the stevia adulteration. The TLC, Colum chromatography, HPLC analytical procedures are sensitive, reproducible, and permit obtaining a sufficient amount of stevia component (Stevioside, Steviol, Rebaudioside-A) for further analytical assessments. Additionally, APCI-MS techniques, FT-NIR spectroscopy analysis, and UV-Vis spectrometry are mainly used for qualitative and quantitative chemical analysis. The humidity, temperature of direct sunlight and quality of packages are the ultimate parameters that impact on the standard of the product.

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