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Nutritional Profile and Health Benefits of Stevia Rebaudiana Bertoni: An Updated Review

NUTRITIONAL PROFILE AND HEALTH BENEFITS OF *STEVIA REBAUDIANA* BERTONI*:* AN UPDATED REVIEW

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ABSTRACT

Aim of this comprehensive review is to document the up-dated data about pharmaceutical potential of *Stevia rebaudiana* Bertoni with special focus on its antioxidant activity. A total of 184 research articles were reviewed and five research engines were used to collect the data. The *S. rebaudiana* (Asteraceae) is indigenous to South America, now cultivated in many countries worldwide to be used as a natural bio-sweetener. Various nutritionally and medically important bioactive compounds such as diterpene glycosides, fatty acids, alkaloids, tannins, saponins, anthraquinones, phenolics, flavonoids, carbohydrates, carotenoids, steroids, polysaccharides and other phytochemicals constitute chemical composition of *Stevia*. Owing to the presence of these substances, *Stevia* possessed antioxidant, anti-inflammatory, antidiabetic, anticancer, antilarvicidal, antibacterial, antimicrobial, antifungal, antiseptic, antifertility, antihyperglycemic and genotoxic properties which adds on to its range of application in food, cosmetics and medicine industries. This review encompasses important bioactivities of *S. rebaudiana* emphasizing on its antioxidant activity along with a glance at its taxonomical, ecological, morphological, chemical, traditional, ethnobotanical and geographic demonstration. The study directs to further exploration of mutagenic, cytotoxic, anticancer, anti-larvicidal and anti-nematicidal effects of *Stevia* to declare its application safe for humans. Experimentation for production of biofuel form steviol extracts is also suggested.

Keywords: Alternate resources, antioxidant property, medicinal plants, ethnopharmacology.

INTRODUCTION

The name of *Stevia* was originated from the spanish botanist name i.e. P. J. Stevus, who was pioneer in studying various species of this genus. Afterward, the M. S. Bertoni discovered sweet taste from the *Stevia* and proposed *S. rebaudiana* in 1899, in recognition of a Paraguayan chemist, Dr. Rebaudi. Later on, two French chemists namely Bridel and Lavieille identified Steviosides and Rebaudioside A from leaves, which were accountable for the sweet taste. The chemical structure of stevioside was framed and articulated as aglycone, steviol with glycoside in 1952 (Abdullateef and Osman, 2011) . During 1970s, rebaudioside was also recognized as greater sweet potential than the stevioside (Ahmad et al., 2020).

Commercially, this species was brought into cultivation in Paraguay during 1964 (Ahmad et al., 2016). In Japan, this plant was doemsticated as a substitute to sweetner when saccharin was banned (Ahmad et al., 2011a). During the Second World War, *Stevia* was used as a sweetener to meet food shortage confronted by Britain. Afterward, the scientific research exposed the usefulness of this species and several countries such as Latin America, Canada, China, Japan, Indonesia, USA started its commercial cultivation (Ahmad et al., 2011b).

The antioxidant compounds along with enzymes like glutathione peroxidase, catalase and superoxide dismutase comprises a powerful antioxidant defense system that breaks down hazardous hydrogen peroxides, hydroperoxides and other free radicals into less harmful molecules and ultimately prevents various human diseases such as atherosclerosis, stroke, cancer, neurodegenration and diabetes. Flavonoids have significant anticancer action on ovarian cancer cells specifically luteolin, myricetin and apigenin (Tavsan and Kayali, 2019). Cellular antioxidants, particularly vitamin E proved efficient against monocyte adhesion, cytotoxicity of oxidized LDL, platelet activation and endothelial dysfunction which cause atherosclerosis and thus decline the rate of coronary artery diseases (Diaz et al., 1997). Bioactive antioxidant compounds such as phenols, flavonoids, tocopherols, phospholipids, carotenoids, ascorbic acid, hydroxycarboxic acids, olive oil resins, glutathione, taurine, selenium, squalenes, sterols, alcohols, vitamins A, E and C, derivatives of cinnamic acids, coumarins and organic acids have ability to scavenge free radical ions. The synthetic antioxidants are being replaced by natural antioxidants in order to prevent oxidation and enhance food quality (Pokorný, 1991; Shahidi et al., 1992; Shahidi, 2000).

Many plants and their extracts are source of natural antioxidants but *S. rebaudiana* plays dual role being source of nonnutritive biosweetener imparting sweet flavor without calories as well as antioxidant agent (Ghanta et al., 2007; Putnik et al., 2020) and can be used as potential alternative to synthetic antioxidant (Mohamed et al., 2017). It possesses higher radical scavenging ability than commercial stevioside (Rao, 2014) since the leave extracts expressed high antioxidant activity than those of BHT and α-tocopherol as standard antioxidants in a comparison test (Elhassaneen, 2019). The ethanolic extract of *S. rebaudiana* described high phenolic content (61.50 mg/g) and radical scavenging ability than standard ascorbic acid thus, advocating its use as natural antioxidant agent (Raut and Aruna, 2017; Sairkar et al., 2009).

a) Taxonomy

Stevia genus consists of 230 species of herbs, subshrubs and shrubs belogs to Asteraceae family (Gupta et al., 2013). Several species of this genus such as *Stevia anisostemma, bertholdii, S. crenata, S. dianthoidea, S. enigmatica, S. eupatoria, S. lemmonii, S. micrantha, S. phlebophylla, S. plummerae, S. rebaudiana , S. salicifolia, S. serrata* and *S. viscida* possess sweetness, however, *S. rebaudiana* possess the optimal level (Bondarev et al., 2010). (Table 1). *Stevia* genus belongs to the family Asteraceae and of which only two species *S. rebaudiana* and *S. phlebophylla* produce sweet compounds (Gantait et al., 2015; Saqib et al., 2015).

b) Habitat

Stevia rebaudiana is well adopted to a wide range of habitats such as grassland, scrub forests, forested mountain slopes, conifer to subalpine forest (Soejarto et al., 1983a) with a favorable temperature ranges of 21°C to 43°C (Gupta et al., 2013).

c) Geographic Distribution

It is native to Paraguay and South America and cultivated in various countries such as Japan, Australia, Brazil, Korea, Mexico, US, Indonesia, India, Bangladesh, Thailand, Tanzania, Canada, Northern Russia, Taiwan, Malaysia, Israel, Ukraine, Philippines, Hawaii and California (Libik-Konieczny et al., 2018; Debnath, 2008). China is the largest producer of *Stevia* with about 13,400 ha area of cultivation with approximately 40,000 tons leaves production every day (Šic Žlabur et al., 2013).

Table 1: Systematic classification of *Stevia*

Kingdom	Plantae
Subkingdom	*Tracheobionta*
Division	*Magnoliophyta*
Subdivision	*Spermatophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Superorder	*Asteranae*
Order	*Asterales*
Family	*Asteraceae*
Sub family	*Asteroidae*
Genus	*Stevia*

d) Vernacular Names

S. rebaudiana is popularly known as honey yerba, honeyleaf, meethi tulsi, sweet chrysanthemum (Carakostas et al., 2008), sweet weed, sugar leaf, sweet leaf (Ghaheri et al., 2017), phawophul (Ho-Dzun et al., 1997) candy leaf (Coggin et al., 2020), madhu patra, sarkara chada, meethi patti, madhu parni, seeni tulsi and oil-kiryata (Singh et al., 2015). Due to the origin in Paraguay and Brazil, this species is known as the sweet herb of Paraguay (Covarrubias-Cárdenas et al., 2018). (Table 2). It is grown in China, Taiwan, Thailand, Korea, Brazil, Malaysia, Canada, Hawaii and California. Rajasthan, Maharashtra, Kerala and Haryana are the main *Stevia* growing states in India.

Table 2: Vernacular names of *Stevia*

Language	Vernacular name	References
Korean	Phalwolphul	(Ho-Dzun et al., 1997)
English	Sweet leaf, candy leaf, sweet honey	(Brandle et al., 1998)
Sanskrit	Madhu patra, sarkara chada	(Singh et al., 2015)
Hindi	Meethi patti, Madhu parni	
Tamil	Seeni tulsi	
Marathi	Oil-kiryata	

e) Botany of Stevia

The *S. rebaudiana* is a perennial, bushy herb that grows up to 2-3 meter height (Upadhyay et al., 2013; Mitra and Pal, 2007) in fertile loamy soil. The roots are cylindrical (Reis et al., 2017), filiform (Singh et al., 2015). The stem is brittle, semi-woody and weak-pubescent at the bottom. The leaves are sessile, elliptical, oppositely arranged with large (4-5 µm) and small (2.5 µm) trichomes. The epidermis of *Stevia* leaf comprised of isodiametric cells underlying the cuticle layer with anomocytic stomata relatively more on the lower side (Bondarev et al., 2010; Abdullateef and Osman, 2011). The flowers are small (15-17 mm), pentamerous, white in color, hermaphrodite, composite, inflouresecnce is sympodial cyme, with small corymbs having two to six florets, while fruit is spindle-shaped, five-ribbed achene with a feathery pappus (Lemus-Mondaca et al., 2012; Yadav et al., 2011) (Figure 1). The flowering season of plant varies from mid of summer to late fall. The plant can be harvested after four months of plantation before starts of flowering that contribute to increased stevioside contents in the leaves (Šic Žlabur et al., 2013). The seeds start germinating in 7 to 10 days but propagation through seeds is not popular due to the weak production and germination rate (Yadav et al., 2011). Besides, cuttings of *Stevia* stem treated with growth hormones are effective means of propagation (Ijaz et al., 2015).

Figure 1: *Stevia rabaudiana* herb

f) Ecological Requirements

This is a short-day plant with the flowering period during January to March in the southern hemisphere. The suitable climates for *Stevia* growth includes semi humid and subtropical regions with annual rainfall between 1500 mm to 1800 mm along with temperature ranges between 21 to 43 °C. The preferable soil types include sandy or loam with pH from 6.5 to 7.5 and 12 hours of exposure to day light (Ijaz et al., 2015) that improves length of internodes, enhances leaf area, dry weight of plant as well as vigorous growth of leaves (Yadav et al., 2011).

g) Laboratory culturing of Stevia

The seed and vegetative propagation methods are not enough to meet the demands of *Stevia* for its commercial application. For the rapid and roduction at large scale, it is necessary to utilize tissue culture techniques. Various studies reported micropropagation of *Stevia* by using stem-tip, nodal region, anther and somatic embryo taken as explants (Gupta, 2013). The explants expressed maximum sweetener constituents when cultured in MS medium accompanied with 6-benzyladenine and indole-3-acetic acid in a quantity of 8.87 mM and 5.71 mM respectively (Sivaram and Mukundan, 2003). Moreover, *Stevia* leaf tissue culture on MS medium was used to determine steviosides content through HPLC analysis. The investigated concentration of stevioside content was 9.6 % (Karim et al., 2015). The light exposure and supplementation of external elicitors improved antioxidant ability as well as phenolic and flavonoid contents of cultured roots of *Stevia* (Alvarado-Orea et al., 2020).

Hairy roots of *Stevia* cultured under different conditions of light and osmotically active substances were able to produce steviol glycosides (Libik-Konieczny et al., 2020). The clonal propagation of *Stevia* was performed on liquid medium. For this purpose *Stevia* explants were cultured in liquid medium comprising of 1.5 mg/L and 0.5 mg/L volumes of BAP and IAA respectively. Regenerated plantlets had reduced root and shoot developing ability as compared to plantlets produced on solid medium but expressed 77 % rate of survival in field conditions (Kalpana et al., 2009). The nodal segments with axillary buds of *Stevia* were cultured in MS medium supplemented with growth hormones for optimization of in vitro regeneration procedure. After 30 days of culturing 2.0 mg/L N-benzyl amino purine (BAP) and 1.13 mg/L indole acetic acid (IAA) were most effective in inducing bud break and shoot proliferation at the rate of 39 microshoots. While for rooting of the shoots of *Stevia* 2.0 mg/L indole butyric acid (IBA) (Debnath, 2008). The rooted plantlets of *Stevia* were treated with growth hormones indol-3-butyric acid (IBA) and alphanaphthylacetamide (ANA) and subjected to natural growth environment for vegetative propagation. They proliferated and spread well in the months from February to July excluding June (Castañeda-Saucedo et al., 2020).

h) Chemical Constituents

The *Stevia* leaves possess ent-kaurene-a diterpene glycoside (stevioside and rebaudiosides) which can be used alternative to synthetic sweetner because of 300 times sweet to the sucrose (Kohda et al., 1976). Besides, various dietray and phytochemical components such as protein,amino acid, fatty acids, alkaloids, glycosides, tannins, saponins, anthraquinones, triterpenes, phenolics, flavonoids, carbohydrates, carotenoids, steroids, polysaccharides, vitamins and minerals have been recorded from this plant (Tadhani and Subhash, 2006a). The phytochemicals produced by *Stevia* includes more than 30 different kinds of steviol glycosides for example rebaudiosides A, B C ,D, E, F, M, steviolbioside, stevioside, dulcoside A and C, with the stevioside and rebaudioside A being major components (Akbari et al., 2017) which are 30 to 320 times more sweet as compared to sucrose (Brandle et al., 1998), heat and pH stable and non-fermentable (Das et al., 2012).
Due to high potency of stevioside (150 to 300 times), it also imparts an undesirable biter-off flavor in products fortified with it (Bhardwaj et al., 2020). Moreover, *in vitro* regenerated roots of *Stevia* can produce secondary metabolites (Reis et al., 2017) and fructans or fructooligosaccharides comprised 46 % of its root reported to have protective potential such as metabolism of lipid, prevention of diabetes (De Oliveira et al., 2011). Along with these phytochemicals, different parts of *Stevia* also contain another effective and important group of polyphenol esters (PPS), chlorogenic acids, which significantly contributed to the hydrophilic antioxidant ability, anti-inflammatory, anti-cancer and anti-diabetic potential of *Stevia* (Myint et al., 2020) (Table 3). The *S. rebaudiana* was subjected to foliar spray of *Moringa oleifera* Lam for seeking enhancing effects on the growth and development of phytochemicals. The results revealed that there was a significant positive effect on the growth and development as well as the production of phytochemicals in *Stevia* and also enhanced its stevioside content considerably (Jain et al., 2020).

a) Traditional Uses

Traditionally *S. rebaudiana* is being used as a non-caloric natural sweetening and antioxidant agent in various products such as soya sauce, pickles, ice-creams, cookies, soft drinks, cosmetics, weight loss products (Mali et al., 2015; Treciokiene and Sostakiene, 2020) and for various medicine and household purposes (Taware et al., 2010). The *S. rebaudiana* can be included in tea, coffee, cooked or baked products, processed food and beverages, jams and jellies, pastries and chewing gums as sweetener (Sairkar et al., 2009). The *S. rebaudiana* extracts are utilized to add sweetening taste in Japan, Korea and some South American countries as well as in soft drinks, soju, sauces, and some other food products (Tadhani and Subhash, 2006a).

Table 3: Phytochemical constituents of *Stevia*

Main group	Constituents	Part	Reference
Polysaccharide	Fructooligosaccharide	Root and leaf	(De Oliveira et al., 2011)
Diterpene glycosides	Stevioside A, rebaudioside A, C, D, E, F, M, steviolbioside, dulcoside A and C, astroinuline, jhanol and 7-acetate.	Leaves, flowers, stem, root	(Upadhyay Sharma and Kumar, 2013; Yadav Kumar and Guleria, 2012; (Darise et al., 1983); (SAKAMOTO et al., 1977)
Sesquiterpenes	β –caryophyllene, trnas-β-farnesene, α-humulene, nerolidol.	Leaves	(Marković et al., 2008)
Alkaloids	Steviamine, stevioside	Leaves, stem , root	(Kumari and Chandra, 2015); (Michalik et al., 2010)
Steroids	Lipid cholesterol, Glucocorticoid	Leaves, stem and root	(Kumari and Chandra, 2015)
Saponins	Steroid saponins	Stem, root and leaf	
Tannins	Tannic acid, gallic acid	Leaves	(Kovačević et al., 2018)
Flavonoids	Querciten, rutin catechin, epicatechin and apigenin.	Leaves, root and stem	(Howlader et al., 2016)
Carotenoids	Lutein	Leaves	(Kovačević et al., 2018)
Phenolics	Protocatechuic acid, gallic acid, cholorogenic acid, caffeic acid, cinamic acid.	Leaves	(Howlader et al., 2016).
Carbohydrates	Glucose, starch	Stem, root, leaves	(Kumari and Chandra, 2015)
Chlorogenic acids	Hydroxycinnamic acid, quinic acid	Root, stem, leaves	(Myint et al., 2020)

In the Canada, France and Turkey, *S. rebaudiana* is consumed as natural flavoring agent and food supplement in beverages, bakery and various other food products (Uçar et al., 2018). *S. rebaudiana* is also used in making ice cream with low calories (Alamprese and Moriano, 2016).

b) Ethnomedicinal Uses

S. rebaudiana is used as dietary supplement in United States. It is used as alternative to sweetner in tea for antidiabetic patients in Bangladesh (Hossain et al., 2010). The use of *S. rebaudiana* as a tea to cure diabetes, to regulate arterial pressure in hypertensive person, milled aerial parts taken as contraceptive by women in Paraguay was confirmed in an ethnobotanical survey (Soejarto et al., 1983b). The soft drinks, sauces and yogurt are sweetened by steviol glycosides in Japan, Korea and Brazil (Alvarado-Orea et al., 2020) (Figure 2).

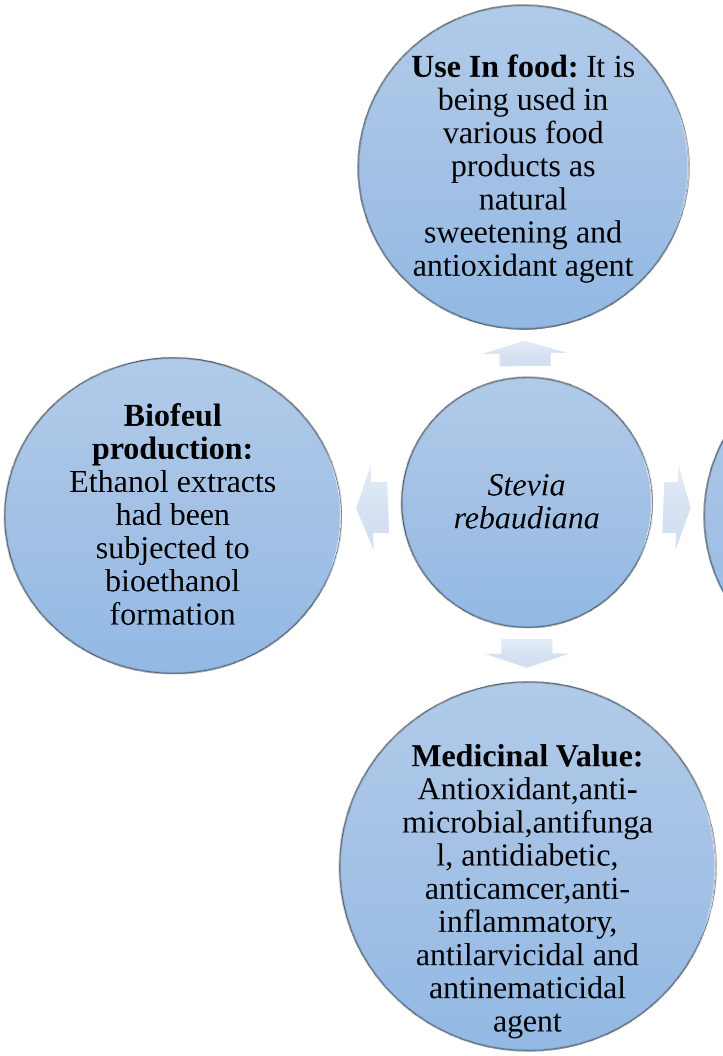

Figure 2. Various applications of *S. rebaudiana*

c) Extraction of Steviosides

The extraction method and solvent type are important parameters affecting the use as antioxidant and sweetener. The solid-liquid extraction at different temperature, microwaves, voltage electric discharge and ultrasound techniques are being used. The best method depends on the utilization of final product as antioxidant or sweetener (Periche et al., 2015). The microwave and ultrasonically assisted extraction of *Stevia* leaves have radical scavenging activities of 91.39 % and 92.40 %, respectively (Yildiz-Ozturk et al., 2015). The oven dried explants of *S. rebaudiana* had maximum antioxidant activity, phenol and flavonoid content, while frozen fresh samples had maximum range of total tocopherol and sugar compounds (Barroso et al., 2016). The ORAC (oxygen radical absorbance capacity) examination of antioxidant ability of *Stevia* extractions obtained from drying method expressed maximum value at 40°C (Lemus-Mondaca et al., 2016). The ultrasound extraction yielded steviosides (50.8 ± 0.1 mg/g) in water solvent while the microwave extracted rebaudioside A (22.7 ± 0.1 mg/g). The pulsed electric technology proved an effective way to get stevioside (44.2 ± 0.1 mg/g) and rebaudioside (22.4 ± 0.3 mg/g) from *Stevia* directing the scaling up of non-conventional technologies to extract antioxidant compounds and valuable compounds from the plant (Carbonell- Capella et al., 2017). The acetone and methanol extracts of *S. rebaudiana* had relatively higher antioxidant capacity as compared to ethanol extraction of *Cassia alata* and *Andrographis paniculata* (Phansawan and Poungbangpho, 2007). The methanolic extract of *Stevia* expressed the IC50 value of 32.765 µg/mL when antioxidant activity was assayed using DPPH method (Raut and Aruna, 2017). The DPPH and FRAP assays used to test methanolic extract advocated the *Stevia* as powerful antioxidant (Najafian et al., 2016).

A significant difference in the yields of steviol glycosides and antioxidants were found according to extraction method. Therefore, a negative correlation occurred between the yield and various extraction treatments. There is no single method for obtaining highest yield in Steviol glycosides and antioxidants (Periche et al., 2015). The concentration of eleven amino acids in *Stevia* dried leaves was 11.70 mg/g and in infusions was in the range of 6.84-9.11 mg/g. The infusion showed high level of alanine, asparagine, leucine and proline amino acids as well as antioxidant compared to the dry leaves. The application of different temperatures had more influence than changing time on the extraction of antioxidant compounds. The infusion at 90 °C for 5 min had 8 out of 11 amino acids, therefore domestic boiling method for some time is best for preparation extract of *Stevia* (Periche et al., 2014). The phosphomolybdenum complex method demonstrated maximum antioxidant activity of purified *Stevia* compound (Mohamed et al., 2017) while a combination of *Rosella* and *Stevia* in a ratio of 1:3 was most stable and effective antioxidant.(Nurkhasanah and Yulianny, 2016). The extraction of stevioside from *Stevia* leaves by enzyme assisted extraction method (Hemicellulose enzyme) resulted in maximum retrieval of stevioside in 1 hour at a temperature range of 60 °C (Puri et al., 2012). The methanol and ethyl acetate fraction from leaves of *Stevia* UEM-13 have maximum antioxidant activity of 93.5 % and 97.32 %, respectively. (Milani et al., 2017a). The alcoholic and BHT (butylhydroxytoluene) extracts from *S. rebaudiana Bertoni* represented considerable antioxidant activity in DPPH and Dot-Blot test (Gopalakrishnan et al., 2006). Using 50 % ethanol for 5 min with ultrasound assisted extraction (UAE) method gave maximum antioxidant activity of *Stevia* preparations (Covarrubias-Cárdenas et al., 2018). Using 50 % ethanol highest phenolic and flavonoid contents as well as antioxidant capacity were determined during solid-liquid solvent extraction of *Stevia* leaves (Medina-Medrano et al., 2019).Three active compounds of *S. rebuadiana* namely; 3-4-dicaffeolylquinic, 3-5-dicaffeolylquinic acids and quercetin-3-O-α-rhamnopyranoside possessed lower antioxidant ability (60 µmol/L) as compared to trolox (Masuda et al., 2006).

The antioxidant activity of *Stevia* investigated by DPPH radical scavenging, ferric thiocyanate (FTC), thiobarbituric acid (TBA), reducing power, total phenol content (TPC), and total flavonoid content (TFC) assays showed a little bit variations in response to different concentrations of applied nitrogen. (Atas et al., 2018). The methods HVED (high voltage electrical discharge), PEF (pulsed electric field) and UAE extractions of steviol content expressed a considerable increase in antioxidant compounds (Barba et al., 2015). The polyphenol and antioxidant content of *S. rebaudiana* was increased using seven different drying methods of freezing, shading, infrared, convective, vacuum, microwave and sun drying while freezing and shade drying resulted in highest and infrared drying in lowest value of polyphenol and antioxidant (Lemus-Mondaca et al., 2018).

BIOACTIVITIES

a) Use as Antioxidant in Food

The *S. rebaudiana* is used as efficient source of natural antioxidants and bio-sweetener in food industry due to presence of important bioactive and nutritive compounds (Gaweł-Bęben et al., 2015; Gasmalla et al., 2014). It is used in coffee, lime, conservation of salmon derived foods, green tea, cookies, functional beverages and fruits etc to enhance phenolic contents and ultimately antioxidant activity of these products (Ortiz- Viedma et al., 2017; Kaushik et al., 2010; Yoo and Hong, 2012; Carbonell-Capella et al., 2015; Criado et al., 2014). The yogurt matrix, was fortified with 0.25 % and 0.5 % freeze-dried *Stevia* extract, preserved the total phenolic content, total solids and antioxidant activity without any effect on the product during the storage (de Carvalho et al., 2019). The antioxidant activity of whey protein isolate (WPI) increased by 80 % when stimulated with 0.2 % *Stevia* fraction (Milani et al., 2017b). Upon mixing with the black tea, it enhanced antioxidant potential of mixture with 326.36 mgGAE/L phenolic content (Budianta et al., 2020a). The green tea, water and coffee were supplemented with selenium using the selenium fortified sweetening leaves of *Stevia* which made the drinks more nutritious for humans (Szarka et al., 2020). The leaves of *Stevia* possessed high antioxidant activity than stems confirmed by phytochemical analysis of four lines for phenolics and flavonoids (Zeng et al., 2013). The hot water extract of *Stevia* stem prevented the oxidation of fish oil more efficiently than its leaf extracts (Yu et al., 2017). The wheat bread sucrose content was replaced by 50 % *Stevia* extract and demonstrated alpha amylase and glucosidase enzyme inhibition and therefore, well suitable to human nutrition (Ruiz-Ruiz et al., 2015). In sardine oil, *S. rebaudiana* extracts effectively repressed hydroperoxide and volatile component formation as well as oxidation (Xi et al., 1998).

The *Stevia* whole leaf powder (4.0 %) or equal quantity of separated preparations of polyphenol and fiber supplementation with diet enhanced the antioxidant ability of rats after one month of feeding (Shivanna et al., 2013). A biological preparation of *Stevia* had 6.7 times more ORAC value than the natural fish sauce (YU et al., 2008). The *Stevia* is suitable to be used in food and nutritional supplement industries as it has no poisonous influence on hepatocellular carcinoma human cells (Bender et al., 2015). The leaves of *S.rebaudiana* growing in Bangladesh had vital potential as natural antioxidants (Jahan et al., 2010). While plants from Paraguay and Spain possessed radical scavenging ability of 46 % and 57 % respectively, as compared to samples from India, Egypt and Greece (Sytar et al., 2015). Two varieties of *Stevia* from Mexico expressed antioxidant ability same as that of trolox (Ruiz et al., 2015). Five varieties of *Stevia* from five different localities of India were taken and compared for their total phenolic and flavonoid contents. Of all varieties, Kangra variety was found to have highest amount of these phytochemicals and ultimately high antioxidant activity (Mandal and Madan, 2013). The super paramagnetic iron oxide nanoparticles with carbohydrate coating green synthesized by *Stevia* extract counteracted the effect of oxidative metabolites (IC50 value of 65 µg/mL). Therefore, these nanoparticles can be potentially used in biomedical applications (Khatami et al., 2019).

b) Antioxidant Activity of Callus Culture of Stevia

The ethanolic extract of callus, regenerated shoots and plantlets of *Stevia* had radical scavenging ability 87.7 %, 86.3 % and 83.5 % respectively, so they have a powerful antioxidant action (Ahmad et al., 2011b). The white, green, blue and red light exposure of leaf explants grown in MS medium supplemented with benzyl adenine (BA) and dichlorophenoxy acetic acid (2,4 D) affected the biomass and biochemical production. The blue light increased the flavonoid and phenolic biochemical formation and green and red light enhanced the DPPH and reducing power activity, whereas white light promoted callus biosynthesis. The colored light used for increased antioxidants production in *Stevia* is promising strategy (Ahmad et al., 2016). The leaf extracts have more antioxidant power than callus extracts (Kim et al., 2011) as the water (9.66 to 38.24 mg) and methanolic (11.03 to 36.40 mg) extracts of leaves have comparatively higher free radical scavenging ability than those of callus (Tadhani et al., 2007). The treatment of methanolic derivatives of leaves and callus of *Stevia* with chlorocholine chloride (CCC) improved their antioxidant ability (Dey et al., 2013). The extracts of spoiled *Stevia* leaves of *ex situ* and *in vitro* grown plants as well as callus culture expressed efficient antioxidant activity to inhibit practically 100 % of DPPH radicals. This increased scavenging power can be interrelated with higher phenolic content (Laguta et al., 2016) as the amount of phenolic compounds contribute to the antioxidant activity of plant (Ahmad et al., 2016).

c) Antioxidant Activity under Various Physiological Conditions

The antioxidant activity of *Stevia* varied according to harvest time, experimental site and crop age (Tavarini and Angelini, 2013), as well as it also seemed to increase at a faster rate under water stress conditions (Srivastava and Srivastava, 2014) and with the application of nitrogen fertilization when provided with 150 kg N ha^{-1} thereby, enhancing the bioactive potential of plants (Barroso et al., 2018; Tavarini et al., 2015). The plant growth retardants (PGRs) such as chlorocholine chloride (CCC) and PBZ (plactobutrazol) have accelerating protective effect on soluble sugar production and antioxidant activity of *Stevia* under abiotic stress. The gibberellin (GA) inhibitors CCC prevented the total steviol glycoside biosynthesis while PBZ and daminozide (DAM) affected vice versa in drought stressed *Stevia* (Karimi et al., 2014). The combination of GA and PBZ treatment increased the photosynthetic pigments, amino acids, proteins and carbohydrates and antioxidant in green house experiment in *S. rebaudiana* (Karimi et al., 2014; Hajihashemi, 2018; Hajihashemi and Ehsanpour, 2014). A combinantion of spermidine and 6-benzyleadenine (2.0 mg L^{-1}), applied on *in vitro* propagated *Stevia* plants, amplified its antioxidant activity (80.6 %) as compared to the control plant (55.3 %) (Khalil et al., 2016).

The *Stevia* can grow well in saline soil conditions along with the accelerated bioactive potential under different levels of salt (Helmy et al., 2016) and its extracts have considerable antioxidant activity in mitochondria, against over radical formation. (Vaško et al., 2014). The exogenous foliar application of KNO_3 (5 g/L) reduced the negative influence of high salinity on the dry leaf biomass production by upto 26 %. The *Stevia* can grow well at 80mMNaCl salinity condition with significant positive influence on chlorophyll, proline and total phenols content by KNO_3 foliar spray (Mahajan et al., 2020; Shahverdi et al., 2020). The foliar spray of three micronutrients boron, iron and selenium on *Stevia* under salinity ranges of 0, 30 mM, 60 mM and 90 mM NaCl increased the root volume, root length and root dry and fresh weight in pots under natural conditions (Shahverdi et al., 2020). The field experiments to study the impact of foliar spray on the yield of *Stevia* leaf and root are to be studied yet.

The effects of irrigation water containing various salinity ranges of 0.7, 1.5, 2.5 and 4 dS/m obtained from various sources such as $Ca(NO_3)_2$, Na_2SO_4, $MgSO_4$, NaCl, $CaCl_2$, $MgCl_2$ on different growth parameters of *Stveia* were determined. Maximum water-yield response (2.15) for Na_2SO_4 whereas minimum value (1.58) for $CaCl_2$ were observed indicating sensitivity of *Stevia* to water stress generated by the osmotic effects of salts. The irrigation water containing Cl is not fit for the cultivation of *Stevia* (Kurunc et al., 2020). Pre-treatment of *Stevia* with fungal (*Piriformospora indica*) and bacterial (*Streptomyces spp.*) extracts enhanced its ability to resist negative effects of salinity by improving its antioxidant ability as well as total phenolic and flavonoid content (Forouzi et al., 2020). Likewise, pre-treatment of melatonin to seed of *Stevia* improved its growth and biosynthesis of steviol glycosides under salinity and stress (Simlat et al., 2020). The leaf extracts of in vitro regenerated and field adapted *S. rebaudiana* explants were shown to have increased level of water soluble antioxidant compounds (Zayova et al., 2013). The potent antioxidant properties of water estractions of *Stevia* leaves can be accredited to their high phenolic (28.70 ± 0.040 mg/g) and flavonoid (7.06 ± 0.041 mg/g) content (Ali et al., 2022). Three polysaccharides pectin EDTA, pectin and hemicellulose of *Stevia* cell wall have DPPH inhibition percentage of 27.66 %, 59.90 % and 23.21 % respectively (Francine et al., 2014).

d) The Genotoxic Activities of Steviol Extracts

The genotoxic agents are carcinogenic compounds which causes mutations of genes ultimately altering the genetic expression of an organism (Josse et al., 2012).The antioxidant compounds have many beneficial effects but their higher concentration in products can be toxic. That is why, *Stevia* has been checked frequently for its toxic impacts, including genotoxicity, in various investigations (Ramos-Tovar and Muriel, 2017). The steviol oral application (1.04 %, 2.08 %, and 3.12 %) for 90 days caused no considerable behavioral, hematological, clinical variations in rats while high doses caused a decline in cholesterol, total protein and albumin in female rats. The results advocate the safety of oral use of steviol content (Zhang et al., 2017). The oxidative damage, cell cycle activity and chromosomal aberrations slightly increased by oral application of steviol glycosides in bone marrow cells of BALB/c rats for 4 weeks. A positive connection was revealed between total oxidative status and the dose(r = 0.65) and mitotic index and dose values (r = 0.74) (Yılmaz et al., 2020).

The rebaudioside A concentration upto 5000 µg/mL was investigated for genotoxicity *in vitro* and *in vivo* and proved to be non-mutagenic in *Salmonella typhimurium* and *E.coli* and in a chromosomal aberration tests in Chinese Hamster V79 cells and in mouse cells. The rebaudioside A upto 750 mg/kg bw was non genotoxic in the micronucleus test of bone marrow in mice as well as in rats at 2000 mg/kg bw. The results advocated rebaidioside A non-genotoxicity at the tested doses (Williams and Burdock, 2009). The *in vitro* studies supported non-mutagenic and non-clastogenic behavior of stevioside and steviol as they expressed no considerable influences in cultured blood lymphocytes taken from a healthy donor (n = 5) (Suttajit et al., 1993). The human lymphocytes were exposed to different levels of saccharin acesulfame-K, aspartame-acesulfame-K, and *Stevia* for 2 hours. The combinatiom of saccharin and aspartame-acesulfame-K were highly genotoxic while acesulfame-K and *Stevia* (0.5 %) were antigenotoxic. The *Stevia* proved to be non genotoxic at 5 %, 0.5 % and 0.05 % concentrations and represented no consumer risk (Silva et al., 2018). The hydroalcoholic extracts of *S. rebaudiana* had significant cytotoxic and genotoxic effects, showing an IC50 values of 98.82 µg/mL and 68.88 µg/mL when tested for their effects on breast cancer cell line (MCF-7) and human fetal lung fibroblasts (MRC-5) respectively (Shokrzadeh et al., 2018). The stevioside exhibited non-cytotoxic activity at an amount of 1.25 g/L when analyzed in an *in-vitro* cytotoxicity assay (Rajab et al., 2009).

The steviol and steviosides revealed non-mutagenic effects in *S.typhimurium* TA strains and *E.Coli* WP2 *uvrA*/pKMlOl (Matsui et al., 1996). The doses of water based *Stevia* extracts at 500mg/kg body weight and stevioside at 800 mg/kg body weight expressed no considerable toxic effects on female reproductive system in Swiss albino mouse (Kumar and

Oommen, 2008). When assayed for its toxicological impact on human peripheral blood lymphocytes, it expressed no genotoxic effects on them and proved safe to use (Uçar et al., 2018). The concerns for mutagenicity of stevioside and rebaudioside A were shown by (Matsui et al., 1996) before the investigations carried out *in vitro* and *in vivo* for genotoxicity of *Stevia* glycosides. A review article written by (Brusick, 2008) and database for non genotoxicity are sufficient to consider the safety of steviol glycosides in food (Urban et al., 2013).

e) Antimicrobial Activity

There are evidences of utilization of steviol contents as antimicrobial agents (Meireles et al., 2006). The *S. rebaudiana* proved its antimicrobial action by preventing the biofilm formation and inhibiting the growth of *Streptococcus mutans* UA159 (Escobar et al., 2020) along with the inhibition of several other microorganisms such as *Vibrio cholera, Bacillus subtilis, Aeromonas hydrophlya, Escherichia coli, Pseudomonas aeruginosa* etc (Nadaf and Naikwadi, 2015). The methanolic extract of *Stevia* had good zone of inhibition for *S. aureus* and *E. coli* as compared to *Parthenium*, *Ginkgo* and azithromycin and cepaxim antibiotics in agar well diffused method (Fazal et al., 2011). The green synthesized zinc oxide nanoparticles of *Stevia* had positive antibacterial effect on *Leishmaniasis major*, *Styphylococcus aureus* and *E. coli* (Khatami et al., 2018). The extracts of *Stevia* obtained through infrared and convective drying methods had potent antimicrobial power against *Listeria innocua* (Lemus-Mondaca et al., 2018). The water, methanol, ethyl acetate, acetone, chloroform and hexane extracts of *Stevia* leaves were inspected for their antimicrobial effects and proved beneficial against microbial activities of several microbes such as *Proteus vulgaris,* Yeast, *Rhizopus oligoporus, Bacillus megaterium, S. aureus, Salmonella typhi, Aeromonas hydrophila, Candida albicans, Cryptococcus neoformans, Micrococcus luteus, Serrstia marcenscens* (Tadhani and Subhash, 2006b; Jayaraman et al., 2008). The petroleum ether extract was highly potent against 10 microorganisms including fungal and pathogenic bacterial specimens (Ghosh et al., 2008). The antimicrobial activity of methanol extracts of *Stevia* leaves was observed under different treatments of nitrogen ranging from 0, 5, 10, 15 to 20 kg ha^{-1} but none of the concentrations made any significant effect in antimicrobial activity (Atas et al., 2018).

The antibacterial activity of leaf extract of *Stevia* prepared in hexane, methanol, ethanol and ethyl acetate and chloroform solvents was reported against the cariogenic bacteria of genus *Streptococcus* and *Lactobacillus*. The zone of inhibition for four species of *Lactobacillus* was larger (12.3-17.5 mm) than *Streptococcus* in case of ethyl acetate and chloroform extracts (Gamboa and Chaves, 2012). The various dilutions of *Stevia* reduced the biofilm formation *S. mutans* and can be used as non-cariogenic natural sweetener (Escobar et al., 2020; Chen et al., 2020). Furthermore, the acetone, ethanol and methanol leaf extract of *Stevia* showed concentration dependent inhibition of bacterial growth against *S. mutans*. The ethanol and acetone extracts were determined to have considerably more inhibition potential as compared to that of aqueous extracts of *Stevia* (Mohammadi-Sichani et al., 2012). The methanolic and ethanolic extract of *Stevia* contained largest concentration of bioactive compounds and presented maximum inhibition against *B. subtilis* and *S. pneumonia* hence both type of extracts can be acknowledged as strong antimicrobial agents (Zohra, 2015).

The phytochemicals present in *Stevia* had made it potential bactericidal agent. Its aqueous and solvent extracts of ethanol, methanol, petroleum ether and chloroform showed minimal bactericidal concentration (MBC) against 49 extended-spectrum beta-lactamases (ESBL) causing uropathogens between 10 mg/mL to 20mg/mL (Raut and Aruna, 2017). The hexane, ethanol, carbon tetrachloride and aqueous extracts of *Stevia* had inhibitory effects on strain of *Staphylococcus epidermidi, S. aureus* as well as on strains of *Pseudomonas aeruginosa*. Of all preparations maximum inhibitory effect on *Styphylococcus epidermidi* was shown by aqueous extracts (84.4 %) (Miranda-Arámbula et al., 2017). The root and leaf extracts of *Stevia* had high antibacterial power and found effective at a value of 500 mg/ml against *B. subtilis* NCIM 2708 and *E.coli* DM 4100 (Singh et al., 2012). The essential oil and crude extract of *Stevia* are natural antibacterial against *B. subtilis, E. coli, P, aureus, Shigella boydii, Salmonella paratyphi* etc with highest and lowest antibacterial index 11.89 ± 0.07mm, 7.24 ± 0.03mm respectively (Siddique et al., 2014).

In another assay of antimicrobial action of *Stevia* leaves extracts *Enterobacter aerogenes* was found more susceptible to the activity of all preparations (Mali et al., 2015). *In vitro* and *in vivo* grown *Stevia* dried leaves extract in chloroform and methanol showed potential antibacterial activity by agar well diffused method against medically important bacteria (Debnath, 2008). The organic flower extracts of *Stevia* were observed to have higher antimicrobial activity than leaf extracts against pathogenic bacterial viz, *B. subtilis, K. pneumonia, P. vulgaris, S. pneumoniae, S. aureus, P. florescence* (Preethi et al., 2011). The methanolic, ethanolic and aqueous extracts of *Stevia* possessed strong antibacterial potential against many bacteria and can be used in pharmaceuticals and preservative substances.

f) Antifungal Activity

The essential oil and various solvent extracts of *Stevia* are antifungal against *A. niger, Candida albicans* and *Saccharomyces cervisaeae* etc. with highest and lowest antifungal index 12.13 ± 0.08mm and 9.13 ± 0.04mm respectively (Siddique et al., 2014). Antifungal potential of *S. rebaudiana* was evidenced from its inhibitory effect on *Fusarium oxysporum* which is harmful to tomato crop. The steviol extracts prevented *F. oxysporum* infection and improved the growth of tomato plants (Ramírez et al., 2020). Another investigation suggested that the antioxidant compounds of *Stevia* also had antifungal action against *Aspergillus flavus, Fusarium verticillioides* and could be used in future to control the aflatoxigenic fungi of maize (Nadaf and Naikwadi, 2015).

OTHER BIOACTIVITIES

a) Antidiabetic Activity

Along with many other medicinal properties *Stevia* also have considerable antidiabetic property that makes it a good natural cure to diabetes. The antidiabetic potential of *Stevia* was proved by an experiment conducted on male and female diabetic patients of different age groups. They were supposed to take dry leaf powder of *Stevia* 1- 3 g in liquids such as in milk, tea and coffee twice a day along with medicines and without medicine. The consumption of *Stevia* powder for 20 days showed a reduction in fasting and post prandial blood sugar thus indicating its effectiveness against type I and type II diabetes (Nema et al., 2020). The *Stevia* being used as an alternative to sucrose for diabetic patients was tested for its sensory acceptance value. A concentration of 0.36 % w/v of *Stevia* and black tea was considered to impart the required sweet taste (Budianta et al., 2020b).

The water extract of *Stevia* had more anti-diabetic potential (IC50 = 8.63 µg/mL) as compared to the manufactured medicine acarbose (IC50 = 13.73 µg/mL) as it contained considerable amount of phenolic and flavonoid contents (Zaidan et al., 2019) and was found effective against α-amylase (17.4 %) and αglucosidase (8.3 %) (Ali et al., 2022). Various studies in which steviol glycosides were administered to animals and humans proved that they caused a reduction in glucagon and postprandial blood glucose levels in diabetic organisms (Kurek and Krejpcio, 2019; Ahmad and Ahmad, 2018).

b) Anticancer activity

The *S.rebaudiana* has a considerable potential against cancer in cell lines such as cervix, pancreatic and colonic as well as against the gene mutations which leads to cancer development (Ahmad et al., 2020). The *Stevia* was tested for its effects on cervix (HeLa), pancreatic (MiaPaCa-2) and colonic (HCT116) cancer cells and proved efficient natural antioxidant being able to scavenge free radicals (López et al., 2016). Moreover, *Stevia* can be used to cure stomach cancer (Meireles et al., 2006).

The *Stevia* products were found efficient against tumor formation specifically that of skin tumor whereas stevioside expressed its impacts on MCF-7 breast cancer cell line by causing apoptotic cell death (Ameer et al., 2020). The leaves of *Stevia* had *in vitro* anti-tumor and cytotoxic potential due to presence of a phutochemical labdane sclareol (Iqbal et al., 2017). Various studies made to observe anti-carcinogenic effects of *Stevia* advocated its role as antitumor agent and the stevioside has also approved as the efficient preventive compound against proliferation of mouse skin carcinogenesis caused by 7,12-dimethylbenz[a]anthracene and 12-O-tetradecanoylphorbol-13-acetate (Jahangir Chughtai et al., 2020; Konoshima and Takasaki, 2002). Two species of *Stevia* were tested for their preventive action against prostate cancer cells and they inhibite proliferation of tumor in human fibroblasts (Martínez-Rojo et al., 2020). In an investigation of ciplatin-induced (CP) nephrotoxicity steviol extracts repressed inflammation, oxidative damage and apoptosis thereby minimizing the risk of kidney injury (Potočnjak et al., 2017).

c) Anti-inflammatory potential

The steviol obtained from *Stevia* are potent to minimize inflammatory activities without exerting any harmful effects (Kurek and Krejpcio, 2019).Owing to its anti-inflammatory action *S. rebaudiana* proved efficient to treat ischaemia/reperfusion injury of testes in rats (Ganjiani et al., 2020). The *Stevia* aqueous extracts were examined for their ability of wound healing and proved beneficial in wounded mice (Das, 2013). The steviosides showed anti-inflammatory properties by down regulating pathways named as nitrogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-kB) (Zou et al., 2020). While determining anti-inflammatory action of extracts of *Stevia* obtained by different drying methods the vacuum and microwave dried extracts inhibited inflammation in arachidonic acid treated mices while microwave, sun and shade dried extracts had significantly inhibited inflammation caused by phorbol 12-myristate 13-acetate (Lemus-Mondaca et al., 2018).

d) Antilarvicidal Activity

The *Stevia* possessed higher larvicidal activity against *Anopheles stephensi* 4^{th} instar larvae and also could be used effectively against the diseases caused due to production of free radicals (Ahmad et al., 2011a). The glycosides of *Stevia* and whole leaf powder had significant growth inhibition impact on *Spodoptera frugiperda* larvae (Lowery, 2017). The leaves of *Morus alba* treated with *Stevia* inulin powder were fed to three groups of larvae of silkworm *Bombax mori* at their last stage. The leaves were fed for three days to first group, for two days to second group and for one day to third group as a food supplementation. Consequently *Stevia* treated leaves of *Morus alba* proved beneficial and enhanced larval metabolism thereby improving their health (Pawar and Khyade, 2017).

e) Anti-nematicidal Activity

Two nematodes *Meloidogyne incognita* and *M. javanica* are the tomato plant parasites causing losses all over the world. The paralysis effect of leaf water extract (LWF) and wood water extract (WWE) of *S. rebaudiana* in Greece was tested on nematodes. The dose was ranged from 5.4-0.042 mg/mL and exposure time was ranged from 1h, 24h and 48h. A significant paralysis was observed in LWE and WWE against second stage juvenile (J2) in vitro. The in vivo experiment exhibited more efficacy of leaves powder compared to the stem. Moreover, a high concentration of *Stevia* ≥ 50g powder/kg was phytotoxic demonstrating use of high inoculum for better results (Ntalli et al., 2020).

f) Use in Medicine

The *Stevia* had positive pharmacological impact on ROS related diseases (Hajihashemi and Geuns, 2013) and can be used in the treatment of liver diseases and diabetes mellitus due to powerful antioxidant properties (Ramos-Tovar and Muriel, 2017) and exerted a positive impact on type 2 diabetic rats improving their insulin and adiponectin levels (Assi et al., 2020). The *S. rebaudiana* had vast range of application in the treatment of chronic diseases such as renal, cardiovascular, hypertension, blood pressure regulation, dental caries and obesity related disorders as well as exhibited anti-tumor and anti-cancerous properties (Jahangir Chughtai et al., 2020; Singh et al., 2015). In Brazil, steviol contents are used in the treatment of diabetes medicine (Patel et al., 2012). Owing to the presence of medically important natural compounds and large scale application in medicines *Stevia* is an effective component of pharmaceuticals having properties like anti-cancer, anti-hypertensive, anti-caries etc (Ahmad et al., 2020).

g) Use in Cosmetics

The *Stevia* extracts and derivatives can be used in cosmetics industry as a natural means of antioxidant as well as antiaging compounds. The addition of *Stevia* in face care products to reduce bitter taste of the constituents is very crucial in cosmetic preparations. The steviol contents are being used to improve the quality of dermatological preparations. The *S. rebaudiana* extract oral use improves the physical appearance of the hair and reduce hair loss (Nadaf and Naikwadi, 2015)**.**

h) Biofuel Production

The cellulose present in the plants can be converted by enzymes to fermentable sugars. The microbes such as fungi and bacteria metabolize these fermentable sugars to bioethanol (Jugwanth et al., 2020). The fungal and bacterial cellulases yielded significant amount of the reducing sugars after hydrolysis of *Stevia* leaves and proved effective to convert biomass into bioethanol (Souii et al., 2020). Supercritical CO_2 and ethanol extraction of *Stevia* were subjected to biomass conversion for bioethanol production and determined as a beneficial feed stock to be used for fuel formation (Coban et al., 2012). Analysis of hydrodistillate oil showed the presence of important essential oil constituents such as spathulenol, caryophyllene oxide, caryophyllene, ledene oxide-(II), b-guaiene, geranyl vinyl ether, indole ,aristolene epoxide silanediol, cyclopentasiloxane, cyclohexasiloxane and estra-1,3, 5(10)-trien-17-ones etc (Siddique et al., 2016).

CONCLUSION AND RECOMMENDATIONS

Stevia rebaudiana is gaining importance due to its wide applications in nutritional, cosmaceutical and pharmaceutical industries due to an increasing trend towards the use of natural products. In this context, a lot of work has been done to explore its medicinal and nutritional properties. This review article is a comprehensive document based on the published material of this species. It highlights the history, use, phytochemistry and nutritional facts along with medicinal, dietary and nutraceutical components. The pharmacological properties revealed that the plant possess antioxidant, anti-inflammatory, antidiabetic, anticancer, antilarvicidal, antibacterial, antimicrobial, antifungal, anti-nematicidal, antiseptic, antifertility, antihyperglycemic, antihypertensive, immune-modulatory and genotoxic properties. Although various studies scripted the non-genotoxic effects of oral applications of steviol extracts, but few results expressed its mutagenic and cytotoxic effects too which require further work. Few studies reported anti-nematicidal, ani-cancer, anti-inflammatory, anti-larvicidal and anti-diabetic potentials, however, there is a need of further detailed studied to declare the use of steviol products, safe to human health. This study also direct to conduct experiments for the production of biofuel from steviol extracts.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHOR CONTRIBUTIONIF wrote original draft. MM supervised whole work. SS, AT and RQ revised, proofread and edited the article.

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