Asian Journal of Chemistry; Vol. 33, No. 12 (2021), 2919-2923

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23393

MINI REVIEW

A Mini-Review on Molecular Docking Studies and Pharmacological Activities of *Stevia rebaudiana*

BINCY RAJ[1,*], SHEENA MERIN THOMAS[2], SUMA VARGHESE[3], M. GNANA RUBA PRIYA[4], SOOSAMMA JOHN[5], PRUDHIVI RAMAKRISHNA[2] and A.R. ABDUL RASHEED[6]

[1]Department of Pharmacognosy, College of Pharmaceutical Sciences, Dayananda Sagar University, Bangalore-560078, India
[2]Department of Pharmacy Practice, College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru-560078, India
[3]Department of Pharmaceutics, College of Pharmaceutical Sciences, Dayananda Sagar University, Bangalore-560078, India
[4]Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences Dayananda Sagar University, Bengaluru-560078, India
[5]Department of Pharmacognosy, East Point College of Pharmacy, Bangalore-560049, India
[6]Department of Pharmacology, College of Pharmacy, Kannur Medical College, Anjarakandy, Kannur-670612, India

*Corresponding author: E-mail: bincympharm@gmail.com

Received: 30 June 2021; *Accepted*: 25 October 2021; *Published online*: 6 December 2021; AJC-20580

Stevia is a small perennial shrub belonging to the Astraceae family with approximately 240 species, which has been used as a natural sweetener. In addition to its sweetening property, it has medicinal values and other uses. Indigenous tribes of South America were using *Stevia rebaudiana* Bertoni for centuries for its medicinal value. Leaves of stevia produce diterpene glycosides (Stevioside and Rebaudiosides), non-nutritive, non-toxic, high-potency sweeteners. The traditional medicinal system is getting more and more appreciation nowadays, but the therapeutic targets for most of these medicines remain unclear, which slows down the novel drug discovery from these natural products. Computational molecular docking studies are effective tools, which are broadly utilized to identify therapeutic targets and interpret molecular aspects of the ligand-protein interactions during drug discovery. Thus, it also enables the extraordinary structural diversity of natural products to be harnessed in an effective manner. The aim of this article is to present a review on the molecular docking studies and pharmacological activities of steviol glycoside isolated from *Stevia rebaudiana*. In this article, the recently published papers about *Stevia rebaudiana* were reviewed, using scientific sites such as Mendeley, PubMed and Google Scholar.

Keywords: *Stevia rebaudiana*, Diterpene glycosides, Molecular docking, Pharmacology, Stevioside, Rebaudiosides.

INTRODUCTION

Stevia is used as a non-nutritive sugar and herbal supplement. It is known as sweet leaf, honey leaf, candy leaf, sweet weed or sweet herbs [1]. Stevia not only has the sweet taste but also maintains the normal sugar level. It is also used as antioxidant, hepatoprotective, antihypertensive, nephron protective, anti-inflammatory agent, *etc*. [2]. The Stevia genus of Asteraceae family comprises 240 species. Stevia obtains its sweetness because of the presence of diterpenes glycosides (steviol glycosides), such as stevioside (4-13%), dulcoside A (0.4-0.7%), rebaudioside A (2-4%) and rebaudioside C (1-2%), and other less abundant compounds including rebaudioside F, rebaudioside B, steviolbioside, steviolmonoside and rubusoside [3]. Food and Drug Administration (FDA) has classified Stevia into the generally recognised as safe (GRAS) category and established an acceptable daily intake (ADI) of 4 mg/kg bw/day for stevia [4].

Widely known species of this family and genus having sweetening property are *S. phlebophylla*, *S. dianthoidea*, *S. anisostemma*, *S. crenata*, *S. bertholdii*, *S. viscida*, *S. enigmatica*, *S. lemmonii*, *S. eupatoria*, *S. micrantha*, *S. rebaudiana*, *S. plummerae*, *S. salicifolia* and *S. serrate*. However, among these, only *S. rebaudiana* has the maximum sweetness intensity [5,6]. Studies have shown that since ancient times, Stevia is used for various purposes worldwide. The Guarani populations of Brazil and Paraguay have utilized Stevia species, mainly *S. rebaudiana*, called as ka′a he′ê (sweet herb) by them, for centuries as medicinal teas for curing ailments such as heartburn and a sweetener in yerba mate [7]. Stevioside, a diterpenoid glycoside, has three glucose molecules and an aglycone (steviol). Apart from stevioside, other sweetening compounds, including rebaudio-

side A, B, C, D, E, steviobioside and ducoside A, were isolated from the Bertoni leaf of *S. rebaudiana*. These isolated diterpenoid glycosides exhibit the same chemical backbone structure (steviol) and different residues of carbohydrate at C_{13} and C_{19} positions [8-10], which is described in Table-1.

Docking studies on *Stevia rebaudiana* leaf extract: Molecular docking has become more and more important tool for drug discovery [11]. In this review, a brief introduction of the molecular docking studies on stevia glycosides from leaf extracts of *S. rebaudiana* and their pharmacological actions is presented. Molecular docking can be effectively used to predict the binding energies and binding modes of protein-ligand complexes and is the common computer-aided drug design method. This tool is also widely used for various research aspects of natural products.

The major glycosides of *S. rebaudiana* exhibit various sweetness potencies related to sucrose, with the sweetest compound of rebaudioside A (250 times more potent) [12]. Mayank & Jaitak [13] performed docking studies by constructing the homology models of T1R3 and T1R2 subunits of sweet taste receptors in humans on natural sweeteners with *S. rebaudiana*. The binding pattern indicated Ans 52, Asn 44, Pro 343, Ala 345, Gly 346, Ile 352, Ala 354, Gly 47, Ser 336, Ser 329, and Thr 326 as the chief interacting amino acid residues for T1R2. Glu 105, Arg 56, Asp 215, Glu 148, Asp 216, Asp 258, Ser 104, Lys 255, Glu 217, Arg 52, and Leu 51 are related to T1R3. Steviol glycoside interacts with amino acids through hydrogen bond formation with the hydroxyl groups of glucose moieties. Rebaudioside A had the optimum binding towards T1R3 and TIR2, with the dock scores of 7.995 and 12.333 kcal/mol, respectively. For rebaudioside E, these scores were 7.841 and 10.658 kcal/mol, respectively, and for rebaudioside D, those were 7.767 and 9.764 kcal/mol. Rebaudioside B, steviolbioside, stevioside and dulcoside also showed binding towards the two receptors.

Stevioside was the most abundant among the steviol glycosides, followed by steviolbioside, rebaudiosides A-F and dulcoside A along with some derivatives observed in trace amounts. Rubusoside is another member of the ent-kaurene glycoside class. Rebaudioside A, with a 9-fold higher sweetness effect than dulcoside A and rebaudioside C, is the most potent sweetener of steviol glycosides. Numerous steviol glycosides, including rubusoside and rebaudioside C, provide a lingering bitter aftertaste with the sweet sensation [13]. Hellfritsch *et al*. [14] comprehensively screened 25 bitter taste receptors in humans and found that two receptors, hTAS2R14 and hTAS2R4, mediate this bitter aftertaste of steviol glycosides. Acevedo *et al*. [15] reported that steviol glycoside presents only one site for orthosteric binding with these receptors. The free binding energy (ΔG binding) of the receptor with steviol glycosides is hT2R14 (r = –0.89) and hT2R4 (r = –0.95).

Deenadayalan *et al*. [16] identified the mechanism of hypoglycemic activity of Stevioside against diabetic's proteins (AKT & PPAR-γ) by using molecular docking analysis. Stevioside has showed binding energy of –9.6 kcal/mol with AKT protein whereas PPAR-γ showed 6.5 Kcal/mol binding energy with stevioside. Three hydrogen bonds have been formed at His 134, Lys 276, Glu 278 when stevioside has been docked with AKT. But stevioside showed a stable complex with PPAR-γ. Moreover, PPAR-γ formed four hydrogen interactions with the amino acids Lys 373, Gln 437, Thr 440 and Glu 448. Dipeptidyl peptidase 4 (DPP 4) play an important role in glucose metabolism [17]. DPP 4 breaks down gut hormones known as incretins. Drugs to treat type 2 diabetes have been developed which inhibit DPP 4, preventing incretin breakdown and prolonging insulin secretion, increasing its effect [18]. Ayachi and colleague have studied the inhibitory action of rebaudioside A and stevioside against DPP 4 using molecular mechanics, molecular dynamics and molecular docking [19]. According to their findings, the total interaction energy of stevioside and rebaudioside A was 1491.86 kcal/mol and 6623.34 kcal/mol, respectively, showing that stevioside exhibits more optimized inhibition of DPP 4 than rebaudioside A.

The *in vitro* α-amylase inhibitory activity of *S. rebaudiana* extracts was investigated by Singla *et al*. [20], they also conducted *in-silico* studies. According to their results, water extract shows the highest α-amylase inhibitory activity. Specifically, these authors reported a docking score of –14.59 kcal/mol for Rebaudioside A with H-bonding contributing 76.31% (–11.21 kcal/mol) to the total binding energy with the receptor site. According to their findings, the essential amino acids participating in the interactions were Tyr 163, Asp 197, His 299 and Asp 300.

Pharmacological importance of *S. rebaudiana*: Medicinal plants are increasingly being adopted alongside conventional therapy in the treatment of acute and chronic diseases, as they have very few side-effects. In particular, phytocompounds and their chemical analogs have been incorporated into numerous clinically useful drugs. This success has prompted extensive research into additional therapeutic agents that can be derived from medicinal plants.

Antidiabetic activity: The hypoglycemic activity of *S. rebaudiana* is well known. In insulin-deficient rats, stevioside can regulate blood glucose levels by improving insulin secretion and utilization. Insulin utilization enhances in rat liver because of the decrease in PEPCK gene expression from the

TABLE-1
RELATIVE SWEETNESS OF STEVIOSIDES

Chemical constituents (9)	R1	R2	Sweetening power (with reference to saccharose) (10)
Stevioside	β-Glc	β-Glc.β-Glc	300
Rebaudioside A	β-Glc	β-Glc.β-Glc.β-Glc	250-450
Rebaudioside B	H	β-Glc.β-Glc.β-Glc	300-350
Rebaudioside C	β-Glc	β-Glc.α Rha.β-Glc	50-120
Dulcosid A	β-Glc	β-Glc. α Rha	50-120

Stevioside's action of hindering gluconeogenesis [21]. On 34 patients with type 2 diabetic mellitus, a double-blind clinical trial was conducted. Compared with the control (sucralose group), in diabetic patients, the consumption of 2% stevia-sweetened tea (for two months, 1-3 times daily) led to considerable changes in HbA1c and FBS levels with no statistically significant variations [22].

Anti-inflammatory activity: In different inflammatory responses, several inflammatory mediators are produced and secreted. Cellular pathways and inflammatory mediators, including chemokines (*e.g*. monocyte chemoattractant protein 1), cytokines (*e.g*. interferons, interleukins and tumour necrosis factor α), potent inflammation-modulating transcription factor nuclear factor κB and eicosanoids (*e.g*. prostaglandins and leukotrienes) are widely studied in combination with human pathological conditions [23]. Potentially suppressed, LPS-mediated IL-1β, TNF-α and IL-6 release provide anti-inflammatory activities of stevioside [24]. LPS-stimulated nuclear factor (NF)-reporter gene expression was inhibited by an ethyl acetate fraction of the water extract of *S. rebaudiana*. Such an NF inhibition was strongly associated with MCP-1 and IL-6 inhibition [25]. These results indicated that stevioside and *S. rebaudiana* prevent inflammation through inhibition of cytokine production in immune, stromal and/or epithelial cells by downregulating NF-κB and MAPK signalling pathways.

Immunomodulatory activity: Stevioside effectively delayed type hypersensitivity and increased hemagglutination antibody titre and phagocytic activity. Stevioside highly increased proliferation in the Con A- and LPS-stimulated T and B cells, respectively [26]. High concentration of stevioside (2-5 mM) and steviol (0.2-0.8 mM) concentrations reduced cell viability in Caco-2, T84, and HT29 cells. In T84 cells, 2 mM stevioside potentiated the release of TNF-α-mediated IL-8. However, 0.01-0.2 mM steviol considerably suppressed the TNF-α-induced release of IL-8 in the three cell lines. In T84 cells, TNF-α-stimulated I kappa B was attenuated by steviol. The immunomodulatory impact of steviol involves NF-kappa B signalling. By contrast, in non-toxic concentrations, only Cl (-) secretion is affected by stevioside [27].

Antioxidant activity: In many human degenerative conditions, such as cancer, ageing, Parkinsons disease and arthritis, reactive oxygen species (ROS) play an important role. A prominent ROS, hydrogen peroxide, causes DNA damage and lipid peroxidation in cells. The antioxidant action of a few natural compounds, including minerals, vitamins, other nonnutrient compounds of plants and polyphenols, inhibit ROS generation or free radical scavenging, which is beneficial to human health [28]. The ethanolic *S. rebaudiana* leaf extract shows stronger antioxidant activity by inhibiting hydroxyl radicals, DPPH, nitric oxide, hydrogen peroxide scavenging and superoxide anion scavenging than standard ascorbic acid. The ethanolic extract of *S. rebaudiana* leaf extract contains some amount of total phenols. These phenols play a key role in antioxidant control [29].

Anti-hypertensive activity: With the tone of total peripheral resistance (TPR) or systemic vasculature and blood volume, mean arterial blood pressure (mABP) changes directly [30]. In the pathological state, arterial hypertension results from an improper relationship between blood volume and vasculature resistance/capacity. Oral administration of the aqueous extract of stevia for 60 days in normal rats led to an increase in *p*-aminohippuric acid, which indicated renal plasma flow, probably by minimizing the renal vascular resistance [31]. Intravenous stevioside administration into dogs with renal hypertension led to a considerable and dose-dependent decrease in blood pressure. In the aortic smooth muscle cells (A7r5 cell line) of cultured rats, in a calcium medium, stevioside dose dependently inhibited the stimulatory impacts of phenylephrine and vasopressin on intracellular Ca^{2+}. Stevioside did not affect Ca^{2+} influx induced by calcium ionophore (A23187), indicating that through Ca^{2+} channel, the antagonistic effect was obtained [32]. A randomized, multicentre, placebo-controlled, double-blind study reported the antihypertensive effects of stevioside on humans. For the stevioside group, the diastolic and systolic blood pressure substantially decreased, and the effect remained persistent for an entire year. Biochemistry parameters of blood, such as glucose and lipid content, did not considerably change. No critical adverse impacts were observed. Quality of life assessment indicated no deterioration [33].

Anti-hyperlipidemic activity: In albino rats, antihyperlipidemic impacts of the aqueous extract of Bertoni leaves of *S. rebaudiana* were reported. The aqueous extract of stevia reduced the body weight gain by minimizing the feed intake in hyperlipidemic rats. Administration of different concentrations of the stevia extract significantly ($P < 0.05$) minimized total cholesterol, low-density lipoprotein, triglyceride and very low-density lipoprotein levels. By contrast, in hyperlipidemic rats, this administration improved high-density lipoprotein levels after eight weeks compared with that in untreated rats [34].

Antimicrobial activity: The aqueous extract of stevia leaves exhibited an activity against *S. aureus* and *B. subtilis*. Methanol extract provided the maximum and minimum inhibition zone against *P. aeruginosa* and *S. aureus* as well as yeast, respectively. Yeast and *B. megaterium* were highly susceptible to hexane extract and ethyl acetate extract, respectively. *B. subtilis* and *A. niger* were least susceptible to hexane extract and ethyl acetate extract, respectively. Hexane extract showed the maximum activity against yeast [35].

Anticancer activity: The apoptosis induction, cytotoxicity and mechanism of steviol's action on human breast cancer cells were studied [36]. In MCF-7 cells, the IC_{50} of steviol was 185 μM. Fluorescence-activated cell sorter analyses revealed the presence of sub-G_0/G_1 peak ($P < 0.05$) along with steviol-mediated G_2/M-phase arrest ($P < 0.05$) in the MCF-7 cells. Similar to 100 μg/mL 5-fluorouracil, steviol intensively inhibited six human gastrointestinal cancer cells. The mitochondrial apoptotic pathway was followed for the inhibition mechanism, which was confirmed by p21 and p53 activation and the increase in the Bax/Bcl-2 ratio. The mechanism for caspase-3-independent apoptotic pathway was also observed. These findings agree with those of miRNA expression analyses. In steviol-treated gastrointestinal cancer cells, the most regulated miRNAs were miR-6088 (log 2 = −2.54) and miR-203a-3p (log 2 = 1.32) in

HCT-116 and miR-23c (log 2 = –2.05) and miR-1268b (log 2 = 19.85) in MKN-45 [37].

Absorption, distribution, metabolism and excretion: Studies on the metabolism and absorption of stevia glycosides in rats have reported that stevioside cannot be readily absorbed by the upper small intestine due to its high molecular weight. However, colon bacteria degrade stevioside, leading to free steviol. A part of this free steviol is absorbed by the colon and transported to the liver. Its part is excreted in faeces. Subsequently, steviol is transformed into its glucuronide derivative in the liver and finally is excreted from the body through urine. The clearance rate of stevioside is lower and higher than that of *p*-amino hippuric acid and insulin, respectively, suggesting that renal tubular epithelium actively secretes steviol glycosides [38-40].

Toxicology: In mice, rats and hamsters, steviol and stevioside show considerably low acute oral toxicity [41]. After intravenous administration of 32.5 µmol/L per kg bw (equivalent to 26 mg/kg, bw) stevioside into pentobarbital-anaesthetised dogs, no considerable renal ultrastructure alteration occurred and no critical changes were observed in any parameters of plasma, whole blood and renal functions. Stevioside does not present any acute extra renal effects (*e.g*. hypoxemia, which can contribute to nephrotoxicity) and direct renal impacts during 6 h after intravenous administration [42]. For two years, groups of 45 female and 45 male inbred Wistar rats were fed diets comprising stevioside (purity, 85%) at the concentrations of 0%, 0.2%, 0.6% and 1.2% (equivalent to 100, 300, and 600 mg/kg bw per day). After 6, 12, and 24 months, from the tail veins of five female and five male rats belonging to each dose group, blood was drawn for clinical biochemical and haematological tests. Food use and consumption, growth, mortality, and general appearance were similar in the control and treated groups. The mean life span of rats administered with stevioside was did not vary from that of the control rats. At any stage of the study, no treatment-related changes were obtained for urinary, haematological or clinical biochemical values. The severity and incidence of neoplastic and non-neoplastic changes were not related to the stevioside concentration in the diet. The no-observed effect level was 1.2%, which was equivalent to 600 mg/kg bw per day. The safety factor of 100 and acceptable daily stevioside intake of 7.9 mg/kg bw per day was suggested by the authors for humans, according to the stevioside consumption of the rats during the initial three months (the average for females and males was 790 mg/kg bw per day) [43]. Groups of 10 female and 10 male one-month-old golden hamsters (*Mesocricetus auratus*) were daily force fed stevioside (purity, 90%) at the concentration of 0, 500, 1000, and 2500 mg/kg bw per day. During the experiment, each female was mated and borne three litters. While lactating (one month) and in late gestation, females received stevioside with drinking water. The female hamsters were mated again two weeks after the offsprings were weaned. No abnormalities were observed in the fertility or growth of the animals of both the sex. All the males successfully and efficiently mated females; the females had normal oestrus cycles of four days and after mating, became pregnant. The number of foetuses, gestation period and number of offsprings of the treatment group were not significantly different from those of the control group [44]. Oral administration of the aqueous extract of stevia leaves (up to 10%) caused no teratogenic effects and no adverse effects on the fertility of the female rats [45]. Recent studies have also confirmed the safety of stevia, showing that steviol glycosides are not carcinogenic, mutagenic, genotoxic [46] and/or teratogenic [47]. For 5 weeks, the rats were fed a diet of 0.5% stevioside or rebaudioside A. Both the compounds showed no potential for increase in the risk of dental caries development [48].

Regulatory status of stevia glycosides: Globally, numerous scientific studies and regulatory organisations have evaluated and reviewed the safety and use of steviol glycosides. The pure extracts of stevia leaves approved for use in beverages and food by >150 countries. In 1995, FDA revised the import alert for stevia extracts and leaves to allow their utilization in dietary supplements as ingredients. In 2007, the Joint FAO/WHO Expert Committee on Food Additives developed specifications and a safe intake level for seven steviol glycosides, which included the minimum purity of 95%. In 2008, FDA responded to a Generally Recognized as Safe (GRAS) notice for the utilization of high-purity Steviol glycosides acquired from stevia leaves in food as a general-purpose sweetener [49]. Due to the current import alert, FDA has evaluated, filed, and not objected to >50 GRAS notices for using of different high-purity steviol glycosides in food as sweeteners [50]. The European Food Safety Authority estimated the safety of steviol glycosides, which was extracted from *S. rebaudiana* of the Bertoni plant leaves, as sweetener and provided their opinion on 10 March 2010. This authority established an acceptable daily intake (ADI), called as steviol equivalents, of 4 mg/kg bw/day for steviol glycosides. Conservative estimates of steviol glycosides exposure in children and adults indicated that ADI is likely exceed the maximum proposed utilization level. Swiss and Hong Kong approvals were obtained between 2011 and 2012 and in 2010. Health Department of Canada, several Asian countries, the Russian Federation and Latin America approved the utilization of steviol glycosides in beverages and foods. During 2014-2016, in several Southeast Asian countries, India, and the Gulf Cooperation Council countries of the Middle East, high-purity steviol glycosides were also approved. In 2015, the Food Safety and Standards Authority of India published the approval for steviol glycosides in India. High-purity steviol glycosides obtained from stevia leaves are approved for their use in major beverage and food categories including dairy, beverages, and tabletop sweeteners [51].

Conclusion

Studies have reported that steviol glycosides present in stevia are not mutagenic, teratogenic or carcinogenic and do not lead to subacute and acute toxicity. Stevia has been used globally as an herbal medicine because it presents no side effects and provides good efficacy. Therefore, to support healthy glucose regulation, steviol glycosides extracted from stevia leaves can replace sugars.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. F. Hossain, M.T. Islam, M.A. Islam and S. Akhta, *Afr. J. Food Agric. Nutr. Dev.*, **17**, 12745 (2017); https://doi.org/10.18697/ajfand.80.16595
2. F. Ahsan, S. Bashir and F.-U.-H. Shah, *Curr. Res. Diabetes Obes. J.*, **13**, 47 (2020); https://doi.org/10.19080/CRDOJ.2020.13.555867
3. M. Segura-Campos, E. Barbosa-Martín, Á. Matus-Basto, D. Cabrera-Amaro, M. Murguía-Olmedo, Y. Moguel-Ordoñez and D. Betancur-Ancona, *Am. J. Plant Sci.*, **5**, 286 (2014); https://doi.org/10.4236/ajps.2014.53039
4. M.F.J. Chughtai, I. Pasha, M.S. Butt and M. Asghar, *Prog. Nutr.*, **21(Suppl.2)**, 210 (2019); https://doi.org/10.23751/pn.v21i2-S.6430
5. D.P. Singh, M. Kumari, H.G. Prakash, G.P. Rao and S. Solomon, *Sugar Tech*, **21**, 227 (2019); https://doi.org/10.1007/s12355-019-00704-1
6. J.C. Ruiz-Ruiz, Y.B. Moguel-Ordoñez and M.R. Segura-Campos, *Crit. Rev. Food Sci. Nutr.*, **57**, 2680 (2017); https://doi.org/10.1080/10408398.2015.1072083
7. R. Lemus-Mondaca, A. Vega-Gálvez, L. Zura-Bravo and A.H. Kong, *Food Chem.*, **132**, 1121 (2012); https://doi.org/10.1016/j.foodchem.2011.11.140
8. V. Chatsudthipong and C. Muanprasat, *Pharmacol. Ther.*, **121**, 41 (2009); https://doi.org/10.1016/j.pharmthera.2008.09.007
9. http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/68/Steviol_glycosides.pdf.
10. C. González, M. Tapia, E. Pérez, D. Pallet and M. Dornier, *Fruits*, **69**, 127 (2014); https://doi.org/10.1051/fruits/2014003
11. X.-Y. Meng, H.-X. Zhang, M. Mezei and M. Cui, *Curr. Comput. Aided-Drug Des.*, **7**, 146 (2011); https://doi.org/10.2174/157340911795677602
12. D.D. Soejarto, E.M. Addo and A.D. Kinghorn, *J. Ethnopharmacol.*, **243**, 112056 (2019); https://doi.org/10.1016/j.jep.2019.112056
13. J.V. Mayank and V. Jaitak, *Phytochemistry*, **116**, 12 (2015); https://doi.org/10.1016/j.phytochem.2015.05.006
14. G.E. Du Bois and I. Prakash, *Annu. Rev. Food Sci. Technol.*, **3**, 353 (2012); https://doi.org/10.1146/annurev-food-022811-101236
15. C. Hellfritsch, A. Brockhoff, F. Stähler, W. Meyerhof and T. Hofmann, *J. Agric. Food Chem.*, **60**, 6782 (2012); https://doi.org/10.1021/jf301297n
16. W. Acevedo, F. González-Nilo and E. Agosin, *J. Agric. Food Chem.*, **64**, 7585 (2016); https://doi.org/10.1021/acs.jafc.6b02840
17. A. Deenadayalan, S. Vijayalakshmi, C.S. Janaki and S. Jayaraman, *Bioinformation*, **17**, 283 (2021); https://doi.org/10.6026/97320630017283
18. R. Godinho, C. Mega, E. Teixeira-De-Lemos, E. Carvalho, F. Teixeira, R. Fernandes and F. Reis, *J. Diabetes Res.*, **2015**, 806979 (2015); https://doi.org/10.1155/2015/806979
19. H. Ayachi, M. Merad and S. Ghalem, *Int. J. Pharm. Sci. Rev. Res.*, **23**, 87 (2013).
20. R. Singla, N. Singla and V. Jaitak, *Nat. Prod. Res.*, **33**, 548 (2019); https://doi.org/10.1080/14786419.2017.1395433
21. T.H. Chen, S.C. Chen, P. Chan, Y.L. Chu, H.Y. Yang and J.T. Cheng, *Planta Med.*, **71**, 108 (2005); https://doi.org/10.1055/s-2005-837775
22. M. Ajami, M. Seyfi, F.A. Pouri-Hosseini, P. Naseri, F. Mahmoudnia, A. Velayati, M. Zahedirad and M. Hajifaraji, *Avicenna J. Phytomed.*, **10**, 118 (2020).
23. A. Azab, A. Nassar and A.N. Azab, *Molecules*, **21**, 1321 (2016); https://doi.org/10.3390/molecules21101321
24. C. Boonkaewwan and A. Burodom, *J. Sci. Food Agric.*, **93**, 3820 (2013); https://doi.org/10.1002/jsfa.6287
25. I.Y. Jeong, H.J. Lee, C.H. Jin, Y.D. Park, D.S. Choi and M.A.J. Kang, *Food Sci. Nutr.*, **15**, 14 (2010).
26. I. Sehar, A. Kaul, S. Bani, H.C. Pal and A.K. Saxena, *Chem. Biol. Interact.*, **173**, 115 (2008); https://doi.org/10.1016/j.cbi.2008.01.008
27. C. Boonkaewwan, M. Ao, C. Toskulkao and M.C. Rao, *J. Agric. Food Chem.*, **56**, 3777 (2008); https://doi.org/10.1021/jf072681o
28. J.C.R. Ruiz, Y.B. Moguel Ordoñez, Á.M. Basto and M.R.S. Campos, *Nutr. Hosp.*, **31**, 1163 (2015); https://doi.org/10.3305/nh.2015.31.3.8043
29. S. Shukla, A. Mehta, V.K. Bajpai and S. Shukla, *Food Chem. Toxicol.*, **47**, 2338 (2009); https://doi.org/10.1016/j.fct.2009.06.024
30. D. De Mers and D. Wachs, Physiology, Mean Arterial Pressure, StatPearls Publishing (2019).
31. M.S. Melis, *J. Ethnopharmacol.*, **47**, 129 (1995); https://doi.org/10.1016/0378-8741(95)01271-E
32. J.C. Liu, P.K. Kao, P. Chan, Y.H. Hsu, C.C. Hou, G.S. Lien, M.-H. Hsieh, Y.-J. Chen and J.-T. Cheng, *Pharmacology*, **67**, 14 (2003); https://doi.org/10.1159/000066782
33. P. Chan, B. Tomlinson, Y.J. Chen, J.C. Liu, M.H. Hsieh and J.T. Cheng, *Br. J. Clin. Pharmacol.*, **50**, 215 (2000); https://doi.org/10.1046/j.1365-2125.2000.00260.x
34. U. Ahmad, R.S. Ahmad, M.S. Arshad, Z. Mushtaq, S.M. Hussain and A. Hameed, *Lipids Health Dis.*, **17**, 175 (2018); https://doi.org/10.1186/s12944-018-0810-9
35. M.B. Tadhani and R. Subhash, *Trop. J. Pharm. Res.*, **5**, 557 (2007); https://doi.org/10.4314/tjpr.v5i1.14633
36. S. Paul, S. Sengupta, T.K. Bandyopadhyay and A. Bhattacharyya, *Nutr. Cancer*, **64**, 1087 (2012); https://doi.org/10.1080/01635581.2012.712735
37. J. Chen, Y. Xia, X. Sui, Q. Peng, T. Zhang, J. Li and J. Zhang, *Oncotarget*, **9**, 26299 (2018); https://doi.org/10.18632/oncotarget.25233
38. G. Brahmachari, L.C. Mandal, R. Roy, S. Mondal and A.K. Brahmachari, *Arch. Pharm.*, **344**, 5 (2011); https://doi.org/10.1002/ardp.201000181
39. E. Koyama, N. Sakai, Y. Ohori, K. Kitazawa, O. Izawa, K. Kakegawa A. Fujino and M. Ui, *Food Chem. Toxicol.*, **41**, 875 (2003); https://doi.org/10.1016/s0278-6915(03)00039-5
40. C. Gardana, P. Simonetti, E. Canzi, R. Zanchi and P. Pietta, *J. Agric. Food Chem.*, **51**, 6618 (2003); https://doi.org/10.1021/jf0303619
41. European Commission Directorate-General XXIV Consumer Policy and Consumer Health Protection Directorate B-Scientific Opinions on Health Matters Unit B3-Management of Scientific Committees II Opinion on Stevioside as a Sweetener (Adopted On 17/6/99) Scientific Committee On Food (1999).
42. Sweetening Agent: Stevioside (WHO Food Additives Series 42); http://www.inchem.org/documents/jecfa/jecmono/v042je07.htm
43. K. Saenphet, S. Aritajat, S. Saenphet, J. Manosroi and A. Manosroi, *Southeast Asian J. Trop. Med. Public Health*, **37(Suppl. 3)**, 203 (2006).
44. J.M.C. Geuns, *Stud. Nat. Prod. Chem.*, **27**, 299 (2002).
45. S. Mathur, N. Bulchandani, S. Parihar and G.S. Shekhawat, *Int. J. Pharmacol.*, **13**, 916 (2017); https://doi.org/10.3923/ijp.2017.916.928
46. M. Matsui, K. Matsui, Y. Kawasaki, Y. Oda, T. Noguchi, Y. Kitagawa, M. Sawada, M. Hayashi, T. Nohmi, K. Yoshihira, M. Ishidate Jr. and T. Sofuni, *Mutagenesis*, **11**, 573 (1996); https://doi.org/10.1093/mutage/11.6.573
47. M. Suttajit, U. Vinitketkaumnuen, U. Meevatee and D. Buddhasukh, *Environ. Health Persp. Suppl.*, **101**, 53 (1993); https://doi.org/10.1289/ehp.93101s353
48. S.K. Goyal, Samsher and R.K. Goyal, *Int. J. Food Sci. Nutr.*, **61**, 1 (2010); https://doi.org/10.3109/09637480903193049
49. J.D. Perrier, J.J. Mihalov and S.J. Carlson, *Food Chem. Toxicol.*, **122**, 132 (2018); https://doi.org/10.1016/j.fct.2018.09.062
50. Commission Regulation (EU) No 1131/2011; https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:295:0205:0211:EN:PDF
51. Arboreal Stevia - Stevia Regulations; https://arborealstevia.com/stevia-regulations/