NATURAL PRODUCT RESEARCH
2019, VOL. 33, NO. 4, 548–552
https://doi.org/10.1080/14786419.2017.1395433

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SHORT COMMUNICATION

Stevia rebaudiana targeting α-amylase: An *in-vitro* and *in-silico* mechanistic study

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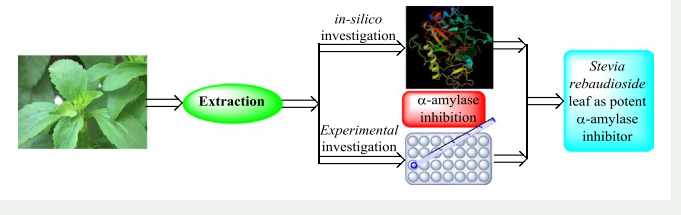
ABSTRACT

Diabetes mellitus (DM) is the fastest growing metabolic disorder in the world. Recently, more attention is paid to the study of natural products due to side effects of synthetic drugs. *Stevia rebaudiana* (Bertoni) is considered an encouraging starting point for the antidiabetic lead development. In the present study, the *in vitro* α-amylase inhibitory activity of the extracts of *S. rebaudiana* is investigated. In order to understand the molecular mechanism and future pharmacophore development, *in silico* study of secondary metabolites isolated from *S. rebaudiana* was carried out. Results indicated that water extract shows highest α-amylase inhibitory activity as compared to other extracts. Moreover, compound 20 (rebaudioside A) which has been previously reported and isolated from water extract showed the impressive binding profile with α-amylase. Therefore, our study suggests that *S. rebaudiana* could be used in the development of therapeutic drugs for the treatment of diabetes.



ARTICLE HISTORY
Received 25 April 2017
Accepted 10 October 2017

KEYWORDS
α-amylase; diabetes mellitus; docking; rebaudioside A; *S. rebaudiana*

1. Introduction

In developed countries, diabetes is the third leading cause of death after heart disease and cancer (El-Ziny et al. 2014). It is a metabolic disorder which is characterised by hyperglycaemia (Samahy et al. 2015). The number of people affected will be 380 million by 2025 as per International Diabetes Federation (Uddin et al. 2014). Current treatment of diabetes includes biguanides, thiazolidinediones, sulphonylureas, and α-amylase inhibitors such as acarbose and miglitol (Uddin et al. 2014). The α-amylase enzyme (EC 3.2.1.1) is a metalloenzyme having Ca^{2+} as a cofactor. It cleaves larger starch molecules into smaller fragments so that

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1395433.

they can easily cross the gut for energy. However, its elevated level consequently leads to an increased plasma glucose concentration causing hyperglycaemia. Thereby, targeting α-amylase is an effective strategy for reducing the postprandial increase of blood glucose level. It has been reported that about 800 species of plants have tremendous antidiabetic potential. *Stevia rebaudiana* is one of the important medicinal plants which belong to Asteraceae family. South American used this medicinal plant as a natural sweetener. Its sweetness is due to the presence of steviol glycosides which are 200–400 times sweeter than sucrose and have low calorific value (Ceunen and Geuns 2013; Singla and Jaitak 2016). The essential oil components extracted from *S. rebaudiana* have been proved to possess potent antimicrobial as well as cytotoxic activity in C-6 (rat glioma cells) and CHOK 1(Chinese hamster ovary cells) in comparison to vinblastine (Agnihotri et al. 2013; Mann et al. 2014). Several reports state its potential therapeutic applications in free radical scavenging (Hajihashemi and Geuns 2013), cancer, hypertension and diabetes (Brahmachari et al. 2011; Mohd-Radzman et al. 2013). Various extracts of *S. rebaudiana* have a therapeutic role in the prevention of diabetes by modulating GLUT-4 (Rizzo et al. 2013) and hepatic gluconeogenesis (Ferreira et al. 2006). It has been reported that *S. rebaudiana* extracts, steviol and stevioside have been proven to possess antidiabetic potential by directly acting on β-cells and producing insulinotropic, glucagonostatic effects and α-amylase inhibiting effects (Jeppesen et al. 2002; Gregersen et al. 2004; Dornadula et al. 2015; Yang and Kong 2016). Moreover, it was found that *S. rebaudiana* extracts are more effective as an antioxidant in down-regulating phosphoenol pyruvate carboxykinase (PEPCK)(Gaweł-Bęben et al. 2015; Wu and Xu 2014). Based on these considerations, the present study is to investigate the α-amylase inhibitory activity of *S. rebaudiana* (Bertoni) and to probe the mechanism of action behind the activity.

2. Result and discussion

2.1. α-Amylase inhibitory activity

Five different extracts of *S. rebaudiana* including petroleum ether (STPE), ethyl acetate (STEA), methanol extract (STM), methanol–water (STMW) and water extract (STW) have been assessed for inhibition of α-amylase. The inhibitory activity was evaluated in the range of 156.25–1000 µg/mL. It was found that there was a dose-dependent increase in percent inhibitory activity against this enzyme (Figure S1). Our findings revealed that the most polar water extract (STW) was most active with IC_{50} 833.68 ± 0.34 µg/mL followed by least polar extract petroleum ether (STPE) with IC_{50} 1033.33 ± 3.6 µg/mL, methanol extract (STM) (IC_{50} 2189.24 ± 3.02 µg/mL) and methanol–water extract (STMW) (IC_{50} 2639.87 ± 1.69 µg/mL). The ascorbic acid which is used as a reference standard worldwide for inhibitory activity have shown IC_{50} 4519.75 + 0.9 µg/mL (Table S1). Thereby, it was evident from the study that *S. rebaudiana* extracts had potent α-amylase inhibitory activity. The STW extract which has shown impressive activity is known to be a source of steviol glycosides including Rebaudioside A-O (Jaitak et al. 2008; Jaitak et al. 2009; Ceunen and Geuns 2013; Singla and Jaitak 2016). The inhibitory activity of stevioside on porcine pancreatic α-amylase was determined and found that 15 g/L reduced the activity by 40% (Pérez-Ramírez et al. 2015). The steviol glycosides consumption has been found not to affect weight, causes improved insulin signalling as well as decreasing atherosclerotic plaque (Geeraert et al. 2010). *In vitro* study indicated that human digestive enzymes could not hydrolyse β-glycosidic bonds (Hutapea et al. 1997).

It is reported that micro-flora degrades steviol glycosides into steviol followed by glucuronide conjugation to form steviol glucuronide which does not enter the bloodstream and gets filtered out by the kidney (Geuns et al. 2006; Geuns et al. 2007; Wheeler et al. 2008). We have found similar results in our study as, stevioside had a weak interaction with α-amylase. To further understand the mechanism behind the activity of extracts containing secondary metabolites, there is a requirement of in-depth analysis of molecular simulation study.

2.2. Molecular simulation study

In-silico study was performed using Maestro software. For the validation of the docking simulation protocol, the co-crystallised ligand present in the protein structure of α-amylase was redocked. The RMSD obtained after superposition of the docked pose of the ligand with crystal structure was 0.02 Å (Figure S2). Secondary metabolites in *S. rebaudiana* that were reported in the literature to act as antidiabetic agents by inhibiting α-amylase were used to conduct the docking protocol. All the compounds isolated from the *S. rebaudiana* have shown better binding affinity in the range of −14.69 to −2.51 kcal/mol as compared to ascorbic acid (−1.72 kcal/mol) which was used as reference standard in *in vitro* inhibitory activity (Table S2). Moreover, Compound 20 (rebaudioside A) has shown best docking score of −14.59 kcal/mol (Figure S3). The binding cavity of the α-amylase is lined by polar amino acids which favour H-bond interaction (Figure S4). Therefore, compounds capable of forming extensive hydrogen bonding were exhibiting superior binding affinity as compared to compounds which have limited polar groups like compound 53, 54 and 55 (Figure S5). Upon analysis of the binding pattern of compound 20, it was found that H-bonding contributes 76.31% (−11.21 kcal/mol) of the total binding energy with the receptor site. The essential amino acids participating in the interactions were Tyr 163, Asp 197, His 299 and Asp 300. Remaining binding energy was attributed by Lipophilic (9.05%) and electrostatic interactions (13.70%). Standard ascorbic acid (66) was also docked and superimposed with compound 20 (Figure S3c) at the receptor site and can be seen that compound 20 occupies the same binding site, where ascorbic acid binds. From the simulation study, it was evident that compounds (21, 27, 28, 30, 33, 34, 36, 37) similar in structure to compound 20, have an impressive binding capacity to the receptor as compared to other compounds. From the structural analysis, it can be concluded that the presence of one glucose moiety at the C-19 position and three glucose moieties at the C-13 position are optimum for binding capacity, increasing or decreasing the glucose moiety causes diminished binding affinity. Furthermore, glucose attached at C-2″ and C-3″ must be in β configuration (compound 20) as compared to α and β configuration, respectively (compound 30). Compound 20 and similar compounds have been reported to be present in high concentration and have been isolated from the aqueous extract of *S. rebaudiana*. Interestingly, we have found that aqueous extract was showing most potent inhibitory activity as compared to other extracts prepared. Dock score, hydrogen binding, lipophilicity and electrostatic energy for all secondary metabolites have been shown in the Table S2.

3. Experimental

All experimental procedures are described in the supplementary materials section (Supplementary S1).

4. Conclusion

Non-insulin-dependent diabetes mellitus has been increasing worldwide at an alarming rate, which makes it crucial to target specifically to prevent systemic side effects associated with current therapy. For attaining this goal, various analogues of antidiabetic drugs have been developed for targeting various targets. Due to this critical need in the current scenario, the focus has been shifted to natural products. Amongst which secondary metabolites from *S. rebaudiana* have been extensively studied for antidiabetic potential. It has a dual role as natural sweetener and prophylaxis for diabetic patients. In the literature, *S. rebaudiana* extracts have been observed to target various causes relating to diabetes. They have been found to help in reducing the activity of α-amylase. From the combined analysis of *in vitro* and *in silico* investigation, we have concluded that aqueous extract of *S. rebaudiana* can serve as a potent source of compounds which have a better therapeutic profile for targeting α-amylase. Further analysis of the binding pattern of Compound 20 with the receptor site has enlightened the key structural requirements for the development of pharmacophore targeting α-amylase. Therefore, our study has provided an in-depth understanding at the molecular level of *S. rebaudiana* extract potential as an antidiabetic agent.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Authors are grateful to University Grants Commission, India for providing financial assistance during the work. Authors are also thankful to the Honourable Vice - Chancellor for providing the necessary facilities at the Central University of Punjab, Bathinda, India.

References

Agnihotri V, Thakur S, Sharma A, Sood S, Kumar R, Pal PK, Gulati A, Singh B. 2013. Essential oil composition and antimicrobial activity of leaves and stem of *Stevia rebaudiana* Bertoni cultivated in the Western Himalaya, India. Indian Perfumer. 57(2):25–50.

Brahmachari G, Mandal LC, Roy R, Mondal S, Brahmachari AK. 2011. Stevioside and related compounds - molecules of pharmaceutical promise: a critical overview. Archiv der Pharmazie. 344(1):5–19.

Ceunen S, Geuns JM. 2013. Steviol glycosides: chemical diversity, metabolism, and function. J Nat Prod. 76(6):1201–1228.

Dornadula S, Elango B, Balashanmugam P, Palanisamy R, Kunka Mohanram R. 2015. Pathophysiological insights of methylglyoxal induced type-2 diabetes. Chem Res Toxicol. 28(9):1666–1674.

El-Ziny MAE-M, Salem NA-B, El-Hawary AK, Chalaby NM, Elsharkawy AA-E. 2014. Epidemiology of childhood type 1 diabetes mellitus in Nile Delta, Northern Egypt – A retrospective study. J Clin Res Pediat Endocrinol. 6(1):9–15.

Ferreira EB, de Assis Rocha Neves F, da Costa MA, do Prado WA, de Araújo Funari Ferri L, Bazotte RB. 2006. Comparative effects of *Stevia rebaudiana* leaves and stevioside on glycaemia and hepatic gluconeogenesis. Planta Medica. 72(8):691–696.

Gaweł-Bęben K, Bujak T, Nizioł-Łukaszewska Z, Antosiewicz B, Jakubczyk A, Karaś M, Rybczyńska K. 2015. *Stevia Rebaudiana* Bert. leaf extracts as a multifunctional source of natural antioxidants. Molecules. 20(4):5468–5486.

Geeraert B, Crombé F, Hulsmans M, Benhabilès N, Geuns JM, Holvoet P. 2010. Stevioside inhibits atherosclerosis by improving insulin signaling and antioxidant defense in obese insulin-resistant mice. Int J Obesity. 34(3):569–577.

Geuns JM, Buyse J, Vankeirsbilck A, Temme EH, Compernolle F, Toppet S. 2006. Identification of steviol glucuronide in human urine. J Agri Food Chem. 54(7):2794–2798.
Geuns JM, Buyse J, Vankeirsbilck A, Temme EH. 2007. Metabolism of stevioside by healthy subjects. Exp Biol Med. 232(1):164–173.
Gregersen S, Jeppesen PB, Holst JJ, Hermansen K. 2004. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabol: Clin Experiment. 53(1):73–76.
Hajihashemi S, Geuns JM. 2013. Free radical scavenging activity of steviol glycosides, steviol glucuronide, hydroxytyrosol, metformin, aspirin and leaf extract of *Stevia rebaudiana*. Free Radicals Antioxidants. 3:S34–S41.
Hutapea AM, Toskulkao C, Buddhasukh D, Wilairat P, Glinsukon T. 1997. Digestion of stevioside, a natural sweetener, by various digestive enzymes. J Clin Biochem Nutr. 23(3):177–186.
Jaitak V, Gupta AP, Kaul VK, Ahuja PS. 2008. Validated high-performance thin-layer chromatography method for steviol glycosides in *Stevia rebaudiana*. J Pharmaceut Biomed Anal. 47(4–5):790–794.
Jaitak V, Bandna BS, Kaul VK. 2009. An efficient microwave-assisted extraction process of stevioside and rebaudioside-A from *Stevia rebaudiana* (Bertoni). Phytochem Anal. 20(3):240–245.
Jeppesen PB, Gregersen S, Alstrup KK, Hermansen K. 2002. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects *in vivo*: studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine. 9(1):9–14.
Mann TS, Agnihotri VK, Kumar D, Pal PK, Koundal R, Kumar A, Padwad YS. 2014. *In vitro* cytotoxic activity guided essential oil composition of flowering twigs of *Stevia rebaudiana*. Nat Prod Comm. 9:715–718.
Mohd-Radzman NH, Ismail WIW, Adam Z, Jaapar SS, Adam A. 2013. Potential roles of *Stevia rebaudiana* Bertoni in abrogating insulin resistance and diabetes: a review. Evidence-Based Complement Alter Med. 2013:1–10.
Pérez-Ramírez IF, Castaño-Tostado E, Ramírez-de León JA, Rocha-Guzmán NE, Reynoso-Camacho R. 2015. Effect of stevia and citric acid on the stability of phenolic compounds and *in vitro* antioxidant and antidiabetic capacity of a roselle (*Hibiscus sabdariffa* L.) beverage. Food Chem. 172:885–892.
Rizzo B, Zambonin L, Angeloni C, Leoncini E, Vieceli Dalla Sega F, Prata C, Fiorentini D, Hrelia S. 2013. Steviol glycosides modulate glucose transport in different cell types. Oxidat Med Cell Longevity. 2013(3):1–11.
Samahy MH, Elbarbary NS, Elmorsi HM. 2015. Current status of diabetes management, glycemic control and complications in children and adolescents with diabetes in Egypt. Where do we stand now? And where do we go from here? Diabet Res Clin Pract. 107(3):370–376.
Singla R, Jaitak V. 2016. Synthesis of rebaudioside A from stevioside and their interaction model with hTAS2R4 bitter taste receptor. Phytochem. 125:106–111.
Uddin N, Hasan MR, Hossain MM, Sarker A, Hasan AN, Islam AM, Chowdhury MMH, Rana MS. 2014. *In vitro* α–amylase inhibitory activity and *in vivo* hypoglycemic effect of methanol extract of Citrus macroptera Montr. fruit. Asian Pacific J Trop Biomed. 4(6):473–479.
Wheeler A, Boileau AC, Winkler PC, Compton JC, Prakash I, Jiang X, Mandarino DA. 2008. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food Chem Toxicol. 46(7):S54–S60.
Wu H, Xu B. 2014. Inhibitory effects of onion against α-glucosidase activity and its correlation with phenolic antioxidants. Int J Food Prop. 17(3):599–609.
Yang X, Kong F. 2016. Effects of tea polyphenols and different teas on pancreatic α-amylase activity *in vitro*. LWT-Food Sci Technol. 66:232–238.