Flavonoid-rich extract of *Chromolaena odorata* modulate circulating GLP-1 in Wistar rats: computational evaluation of TGR5 involvement

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Abstract

Chromolaena odorata is a major bio-resource in folkloric treatment of diabetes. In the present study, its anti-diabetic component and underlying mechanism were investigated. A library containing 140 phytochemicals previously characterized from *C. odorata* was generated and docked (Autodock Vina) into homology models of dipeptidyl peptidase (DPP)-4, Takeda-G-protein-receptor-5 (TGR5), glucagon-like peptide 1 (GLP1) receptor, renal sodium dependent glucose transporter (SGLUT)-1/2 and nucleotide-binding oligomerization domain (NOD) proteins 1&2. GLP-1 gene (RT-PCR) modulation and its release (EIA) by *C. odorata* were confirmed in vivo. From the docking result above, TGR5 was identified as a major target for two key *C. odorata* flavonoids (5,7-dihydroxy-6-methyl-2-pyrone and homoesperetin-7-rutinoside); sodium taurocholate and *C. odorata* powder included into the diet of the animals both raised the intestinal GLP-1 expression versus control ($p < 0.05$); When treated with flavonoid-rich extract of *C. odorata* (CoF) or malvidin, circulating GLP-1 increased by 130.7% in malvidin-treated subjects (0 vs. 45 min). CoF treatment also resulted in 128.5 and 275% increase for 10 and 30 mg/kg *b.w.*, respectively. Conclusions: The results of this study support that *C. odorata* flavonoids may modulate the expression of GLP-1 and its release via TGR5. This finding may underscore its anti-diabetic potency.

Keywords *C. odorata* · Flavonoids · TGR5 · GLP-1

Introduction

The International Diabetes Federation Africa (IDFA) had reported that 14 million Africans are diabetic, with a projection of 34.2 million in 2040 (Foundation 2016). In 2015, alone, 321,000 diabetes-related deaths were recorded. Given the socioeconomic impact of diabetes, a rapid and highly

scalable solution in tackling this epidemic is imperative. Given that most countries in Africa are ranked as low- and medium-income countries, a cost-effective option is an absolute must. Treatment strategy for Type-2 diabetes relies predominantly on improved metabolic status of the patient (Scheen 2003; Scheen and Paquot 2015) while type-1 diabetes is amenable to insulinotropic drugs (Atkinson 2012; D'Alessio 2010). Current pharmacological intervention is rather inaccessible to patients in low- and medium-income demography due to socioeconomic constraints; thus promoting an argument for alternative therapeutic options if current epidemiology and future projections must be averted.

According to folklore, one of the most effective treatment options for diabetes is extract of *Chromolaena odorata* L. which has been backed up by some scientific evidence (Adedapo et al. 2016; Onkaramurthy et al. 2013). Studies linking specific *C. odorata* phytochemicals to anti-diabetic actions are still scarce; and may delay drug-development plans. This study benefitted from previous phytochemical characterization efforts on *C. odorata* and the current understanding of clinical and experimental targets for anti-diabetic

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drugs. In all, a total of 140 phytochemicals were curated and computationally screened against anti-diabetic targets. Dipeptidyl peptidase (DPP)-4 (Barnett 2006), Takeda-G-protein-receptor-5 (G protein-coupled bile acid receptor, TGR5) (Genet et al. 2009), glucagon-like peptide 1 (GLP1) receptor (Drucker and Nauck 2006), renal sodium dependent glucose transporter (SGLUT)-1/2 (Meng et al. 2008) and nucleotide oligomerization domain (NOD)-1/2 (Shiny et al. 2013) were chosen as they represent pharmacological targets for sitagliptin, 3-(2-chlorophenyl)-N-(4-chlorophenyl)-N,5-dimethyl-4-isoxazolecarboxamide, GLP1, ertugliflozin, and GSK717, respectively, which have proven anti-diabetic potencies.

From the computational studies, 5,7-dihydroxy-6-4-dimethoxyflavanone and homoesperetin-7-rutinoside were identified as putative anti-diabetic compounds from *C. odorata* targeting TGR5. Since both compounds are of flavonoid class, total flavonoid was isolated, validated and tested in vivo for serum GLP-1 release. Since GLP-1 is insulinotropic, flavonoids of *C. odorata* may therefore represent another therapeutic strategy for managing diabetes (Lokman et al. 2013; Oh 2015; Shen et al. 2012).

Experimental

Library generation, docking and scoring

Previously identified and characterized compounds of *C. odorata* were retrieved from PubChem using ChemAxon suite (<https://www.chemaxon.com>). The library was docked (Autodock Vina)(Seeliger and de Groot 2010) into the active sites of the homology models (<https://swissmodel.expasy.org/>) (Schwede et al. 2003) of DPP-4 (Barnett 2006), TGR5 (Genet et al. 2009), GLP-1 receptor (Drucker and Nauck 2006), SGLUT-1/2 (Meng et al. 2008) and NOD-1/2 (Shiny et al. 2013). The templates for the model were carefully selected to reflect inhibitor-bound conformation in DPP4 (template = 6B1O, DPP4 in complex with vildagliptin analog, 1.91 Å), SGLUT-1/2 (template = 3dh4, Crystal Structure of Sodium/Sugar symporter with bound Galactose from vibrio parahaemolyticus, 2.70 Å) (Faham et al. 2008) and NOD-1/2 (template = 4JQW, Crystal Structure of a Complex of NOD1 CARD and Ubiquitin, 2.9 Å) (Ver Heul et al. 2014) while TGR5 (template = 3QAK, agonist bound structure of the human adenosine A2a receptor, 2.7 Å resolution) (Xu et al. 2011) and GLP-1 receptor (template = 5NX2, GLP-1R in complex with a truncated peptide agonist, 3.7 Å resolution) (Jazayeri et al. 2017) were modeled in active states. In each case, the binding site is defined by 25 cm by 25 cm by 25 cm rectangular box around the mean coordinate of the co-crystallized ligand and in case of NOD without ligand, Nodinitb-1 (ML130, CID 1088438)

(Kecek Plesec et al. 2016) retrieved from the PubChem database was docked into the NOD using the BSP-SLIM portal as previously reported (Omotuyi and Ueda 2015). Lamarckian genetic algorithm (LGA) in AutoDock was used to generate conformations of the *C. odorata* compounds. Ten docking runs were performed, with an initial population of 150 random individuals and a maximum number of 2,500,000 energy evaluations as successfully implemented in previous studies (Morris et al. 2009).

GLP-1 gene expression

All protocols related to animal studies were approved by the Animal Ethics Committee of Centre for Research and Development (CRD), Adekunle Ajasin University, Akungba-Akoko. *C. odorata* powder ($n = 5$) and sodium taurocholate (Sigma-Aldrich, $n = 5$) were formulated into rat feed (5%, feed 95%), and compared with the control (feed = 100%, $n = 3$). The bitter taste of the treatments was masked using locally sourced honey (100 ml/100 g formulated feed and control group). Animals were allowed to feed ad libitum for 14 h followed by 8 h feed withdrawal. RNA was isolated from the proximal end of the ligated ileum using TRIzol Reagent (ThermoFisher Scientific). DNA contaminant was removed following DNase I treatment (ThermoFisher Scientific) following manufacturer's protocol. Purified DNA-free RNA was converted to cDNA immediately using ProtoScript® First Strand cDNA Synthesis Kit (NEB). PCR amplification was done using OneTaq® 2X Master Mix (NEB) using the following primer set: (GLP-1-Forward-primer-5'-TCCCAA AGGAGCTCCACCTG-3'/Reverse-primer-5'-TTCTCCTCC GTGTCTTGAGGG-3'; β -Actin-(control)- β -Actin-Forward-primer-5'-GTCGAGTCCGCGTCCAC-3'/Reverse-primer-5'-AAACATGATCTGGGTCATCTTTTCACG-3'). GLP-1. Representative snapshot of reverse transcription polymerase chain reaction-agarose gel electrophoresis data of Na-taurocholate (Na-T) and *C. odorata* fed Wistar rats. The band density (Image-J)(Schneider et al. 2012) is plotted as a bar graph (mean $\pm$ SEM, $n = 3-5$).

Flavonoid isolation, characterization, and serum GLP-1 estimation

Flavonoids were isolated from fresh samples of *C. odorata* using HCL (1%, v/v) as previously documented (Omotuyi et al. 2013) and purified using DOWEX-50 column (Raman et al. 2004) resulting in *C. odorata* flavonoids (CoF). CoF HPLC characterization has been described elsewhere (Mattila et al. 2000). Serum GLP-1(EIA, according to the manufacturer's protocol) was assayed before (0 min) and after oral administration (45 min) of CoF ($n = 5$), and compared to malvidin chloride (MaL,

$n = 5$) (Sigma-Aldrich) and untreated control animals ($n = 3$) as previously described (Phuwamongkolwivat et al. 2014).

Results and discussion

Dysregulated glucose homeostasis underlies diabetes mellitus and its microvascular complications. Glucose homeostasis may be altered by autoimmune destruction of pancreatic beta cells or insulin resistance. Therefore, diabetic patients have benefitted from exogenous insulin, treatment strategies that improve endogenous insulin production, reduce obesity and restores energy balance. GLP-1 secretagogues (acting via TGR5 activation) (Cao et al. 2016) and GLP-1 analogs are particularly interesting in clinical settings as they enhance the biological actions of GLP-1 such as promoting β cells proliferation and function (insulin synthesis and GLP-1 receptor-mediated insulin secretion), suppressing gut motility and appetite. GLP-1 is short-lived, rapidly metabolized by serum DPP-4 (Beck-Nielsen 2012; Lee 2016; Yu et al. 2015). Another clinically useful strategy is to elongate GLP-1 by inhibiting DPP-4 (Yu et al. 2015). Recently, NOD-like receptors (NOD1 and NOD2) are now recognized as critical factors in HFD (high-fat diet)-induced pancreatic inflammation and insulin intolerance (Schertzer et al. 2011) and the clinical application of its antagonists is now under intensive investigation (Moreno and Gatheral 2013). Another clinical target for diabetes is the sodium–glucose co-transporter (SGLT)-2 (Mudaliar et al. 2015). Inhibitors of SGLT2 prevent renal glucose reabsorption enhances urinary glucose

excretion with concomitant reduction in plasma glucose levels (Fig. 1).

Identification of TGR5 as *C. odorata* phytocompounds target

The first question in this study is which of these targets may explain the anti-diabetic potency of *C. odorata*? To answer this, a library of 140 phytocompounds was created for *C. odorata* (supplementary information 1) based on previous reports of isolation and structural characterization. Docking results for each targets detailed above are represented as a heat map (Fig. 2a). Although, several phytochemicals of *C. odorata* have high affinity (-9.0 to -12.0 kcal/mol; yellow to red spectrum) for TGR5, GLP1R and SGLT1, It is of interest to note that 5,7-dihydroxy-6-4-dimethoxyflavanone and homoesperetin-7-rutinoside are further interest as they showed the lowest binding energy overall for TGR5; calculated as -11.5 and -11.0 kcal/mol, respectively, compared with -9.4 kcal/mol for 6 α -Ethyl-23(S)-methylcholic Acid (INT-777) a well-studied TGR5 agonist. To validate the computational data obtained for TGR5, 45 agonists with EC_{50} records were downloaded from the ChEMBL database, prepared and docked into the TGR5 model used for *C. odorata* screening. A line graph of experimental p_{50}^{EC} versus docking score showed the equation: Docking score = $-0.84 \times (p_{20}^{EC} \text{ value}) - 1.5$ with the correlation coefficient (r^2) = 0.75 (Fig. 2b). Next, the binding poses were studied to determine the residues involved in TGR5 interaction. INT-777 relied on hydrophobic interaction with Phe-96, Tyr-89, Leu-141, Leu-244, Leu-266, Leu-71 and hydrophobic interaction with Asn-77 (Fig. 3a). The fused rings of 5,7-dihydroxy-6-4-dimethoxyflavanone is stabilized by hydrophobic interaction with Leu-262/266

Fig. 1 Graphical abstract. The contribution of pancreas, intestinal L-cell, kidney and blood factors in the development of novel anti-diabetic agents

Fig. 2 Heat map representation of docking result. The free energy of binding of phytochemicals of *C. odorata* docked into the substrate-binding sites of DPP4, TGR5, GLP1R, SGLT1, SGLT2, NOD1 and NOD2 are represented as heat map. (The scale is a spectrum from

pink (0 kcal/mol) to red (− 12 kcal/mol). **b** The correlation plot of pEC50 (experimental, CHEMBL5409) versus docking score (*Auto-dock Vina*)

residues. The detached A-ring forms hydrophobic interaction with Pro-164 and Leu-168. Ser-156 and Tyr-240 may also play key roles in stabilizing the interaction between TGR5 and 5,7-dihydroxy-6-4-dimethoxyflavanone (Fig. 3b). Homoesperetin-7-rutinoside tends to utilize all the residues highlighted in INT-777 and 5,7-dihydroxy-6-4-dimethoxyflavanone complexes and may also benefit from enhanced hydrophilic interaction resulting from the presence of rutinoside residue (Fig. 3c). While further experimental study was focused on TGR5 effects, it is worthy of note to add that homoesperetin-7-rutinoside also demonstrated low binding energy for GLP1R (− 11.0 kcal/mol) and quercetin 3-O-rutinoside show a low binding energy for SGLT1 (− 11.0 kcal/mol) but not SGLT2 (− 9.8 kcal/mol, binding pose not shown).

Diet-included *C. odorata* alters GLP-1 gene expression in the ileum

If TGR5 is indeed a key target of *C. odorata* phytochemicals, then it should promote GLP-1 gene expression profile, therefore, *C. odorata* powder and sodium taurocholate (Na-T) were introduced into the feed of the subjects as described in the experimental section followed by determination of GLP-1 gene expression in proximal end of the ligated ileum. In the treatment groups, GLP-1 was significantly upregulated ($n = 3-5$ animals, $p > 0.05$ vs. untreated control) but no statistically significant difference was observed between sodium taurocholate and *C. odorata* treatments (Fig. 4). This finding further lends credence to the anti-diabetic effects of dietary flavonoids and further reveals

Fig. 3 Binding poses of select *C. odorata* phytochemicals in the orthosteric site of TGR5. 6 α -Ethyl-23(S)-methylcholic Acid (INT-777, cyan stick representation), a known TGR5 (green cartoon) agonist was docked into TGR5 model using BSP-SLIM method and re-docked using Autodock Vina for validation (a). 5,7-dihydroxy-

6-4-dimethoxyflavanone (Fig. 2b; pink stick representation) and Homoesperetin-7-rutinoside (Fig. 2c; yellow stick representation) were docked (Autodock Vina) into TGR5 using INT-777 as reference ligand. Residues participating in the interaction of each ligand are displayed on top of each complex

Fig. 4 Intestinal GLP-1 expression in *C. odorata* fed Wistar rats. Representative snapshot of reverse transcription polymerase chain reaction-agarose gel electrophoresis data of Na-taurocholate (Na-T) and *C. odorata* fed Wistar rats. The band density (Image-J) (Schneider et al. 2012) is plotted as a bar graph (mean $\pm$ SEM, $n = 3-5$)

a possible mechanism via enhanced GLP-1 production, insulin secretion, pancreatic β -cells proliferation. Ultimately resulting in hypoglycemia, reduced insulin resistance and increased glucose uptake in skeletal muscle and white adipose tissue (Babu et al. 2013). The result is consistent with previous findings that phenolics from berries upregulated insulin-like growth factor 1 receptor, insulin-like growth factor 2, insulin-like growth factor binding proteins, vascular endothelial growth factor (VEGF) and GLP-1 genes in iNS-1E cells (Johnson and de Mejia 2016).

C. odorata flavonoid improves plasma GLP-1

5% *C. odorata* in rat feed is a complex mixture of several phytochemicals and increased in GLP-1 gene expression may not be due to flavonoids alone. Therefore, heterogeneous flavonoids from fresh samples of *C. odorata* (CoF) were isolated as described previously (Omotuyi et al. 2013) and purified (Raman et al. 2004). Flavonoids

Fig. 5 HPLC-DAD profile of CoF: HPLC chromatogram of *C. odorata* flavonoid-rich extract following a 60 min-elution at 1 mL/min flow rate in acetonitrile, 0.1% HCOOH in water. Detector was scanned between 200 and 500 nm

from *C. odorata* absorbed at 230/530 nm following HPLC fractionation (Fig. 5). CoF was compared with malvidin chloride (MaL) and untreated control animals for plasma GLP-1 release as previously described (Phuwamongkol-wiwat et al. 2014). Blood was drawn from animals (0 min, female Wistar rats, 8 weeks old, $n = 3$) previously fasted for 24 h and following 1 mL oral doses (0 (water, control), 30 (MaL), 10, 30 (CoF) mg/kg *b.w.*) of treatment materials after 45 min post administration. GLP-1 data showed no significant difference in the circulating GLP-1 in the pre-treated animals, but following MaL (30 mg/kg), circulating GLP-1 levels increased by 130.7% (vs. water control). CoF treatment resulted in 128.5 and 275% increase for 10 and 30 mg/kg *b.w.*, respectively, in comparison with the water-treated control (Fig. 6). Post-treatment, circulating GLP-1 is significantly higher in CoF-treated (30 mg/kg, *b.w.*) group compare with the MaL-treated animals (Adjusted P value = 0.04, inset, Fig. 6). This data lend credence to the previous reports that non-nutritive components of plant especially the flavonoid class have GLP-1 secretory properties. The authors specifically identified delphinidin 3-rutinoside (D3R) as GLP-1 secretagogue in GLUTag

L cells (Kato et al. 2015). Furthermore, aromatic ring of flavonoids containing three hydroxyl, two methoxyl moieties, or rutinoside may be essential for GLP-1 stimulation. Although this study relied on computation for proposing the plausible target for *C. odorata* flavonoids, Kato et al. (Kato et al. 2015) suggested that D3R stimulated GLP-1 secretion in GLUTag cells via GPR40/120-mediated Ca^{2+} -CaMKII pathway.

Conclusion

This study investigated the putative anti-diabetic principle in *C. odorata* and its underlying mechanisms using in silico and in vivo experiments. 5,7-dihydroxy-6-4-dimethoxyflavanone and homoesperetin-7-rutinoside are the major *C. odorata* compounds with high affinity for TGR5. Normal rats fed *C. odorata*-based food or administered flavonoid-rich extract of *C. odorata* showed increased blood GLP-1 release compared with their control littermates. Interestingly, the results demonstrate that *C. odorata* may

Fig. 6 Plasma GLP-1 dynamics in anthocyanin-enriched *C. odorata* extract-treated Wistar rats. The line graph represents circulating GLP-1 in pre- (0 min) and post-treated (45 min) animals. Post-

treatment values are also represented as bar graphs (mean $\pm$ SEM, $n = 3-5$) and subjected to ANOVA analysis (inset)

represent key bio-resource for the development of cheap anti-diabetic drugs.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Adedapo AA, Ogunmiluyi IO, Adeoye AT, Ofuegbue SO, Emikpe BO (2016) Evaluation of the medicinal potential of the methanol leaf extract of *Chromolaena odorata* in some laboratory animals. *J Med Plants* 4:29–37
- Atkinson MA (2012) The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harb Perspect Med*. <https://doi.org/10.1101/cshperspect.a007641>
- Babu PV, Liu D, Gilbert ER (2013) Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *J Nutr Biochem* 24:1777–1789
- Barnett A (2006) DPP-4 inhibitors and their potential role in the management of type 2 diabetes. *Int J Clin Pract* 60:1454–1470
- Beck-Nielsen H (2012) Glucagon-like peptide 1 analogues in the treatment of type 2 diabetes mellitus. *Ugeskr Laeger* 174:1606–1608
- Cao H, Chen ZX, Wang K, Ning MM, Zou QA, Feng Y, Ye YL, Leng Y, Shen JH (2016) Intestinally-targeted TGR5 agonists equipped with quaternary ammonium have an improved hypoglycemic effect and reduced gallbladder filling effect. *Sci Rep* 6:28676
- D'Alessio DA (2010) Taking aim at islet hormones with GLP-1: is insulin or glucagon the better target? *Diabetes* 59:1572–1574
- Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *The Lancet* 368:1696–1705
- Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, Abramson J (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na^+ /sugar symport. *Science* 321:810–814
- Foundation ID (2016) Executive summary in IDF diabetes. *Atlas*
- Genet C, Strehle A, Schmidt C, Boudjelal G, Lobstein A, Schoonjans K, Wagner A (2009) Structure–activity relationship study of betulinic acid, a novel and selective TGR5 agonist, and its synthetic derivatives: potential impact in diabetes. *J Med Chem* 53:178–190
- Jazayeri A, Rappas M, Brown AJH, Kean J, Errey JC, Robertson NJ, Fiez-Vandal C, Andrews SP, Congreve M, Bortolato A, Mason JS, Baig AH, Teobald I, Dore AS, Weir M, Cooke RM, Marshall

- FH (2017) Crystal structure of the GLP-1 receptor bound to a peptide agonist. *Nature* 546:254–258
- Johnson MH, de Mejia EG (2016) Phenolic compounds from fermented berry beverages modulated gene and protein expression to increase insulin secretion from pancreatic beta-cells in vitro. *J Agric Food Chem* 64:2569–2581
- Kato M, Tani T, Terahara N, Tsuda T (2015) The anthocyanin delphinidin 3-rutinoside stimulates glucagon-like peptide-1 secretion in murine GLUTag cell line via the Ca^{2+} /calmodulin-dependent kinase II pathway. *PLoS ONE* 10:e0126157
- Kecek Plesec K, Urbancic D, Gobec M, Pekosak A, Tomasic T, Anderluh M, Mlinaric-Rascan I, Jakopin Z (2016) Identification of indole scaffold-based dual inhibitors of NOD1 and NOD2. *Bioorg Med Chem* 24:5221–5234
- Lee CY (2016) Glucagon-like peptide-1 formulation—the present and future development in diabetes treatment. *Basic Clin Pharmacol Toxicol* 118:173–180
- Lokman FE, Gu HF, Wan Mohamud WN, Yusoff MM, Chia KL, Ostenson CG (2013) Antidiabetic effect of oral borapetol B compound, isolated from the plant *Tinospora crispa*, by stimulating insulin release. *Evid Based Complement Alternat Med* 2013:727602
- Mattila P, Astola J, Kumpulainen J (2000) Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *J Agric Food Chem* 48:5834–5841
- Meng W, Ellsworth BA, Nirschl AA, McCann PJ, Patel M, Girotra RN, Zahler R (2008) Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. *J Med Chem* 51:1145–1149
- Moreno L, Gatheral T (2013) Therapeutic targeting of NOD1 receptors. *Br J Pharmacol* 170:475–485
- Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem* 30:2785–2791
- Mudaliar S, Polidori D, Zambrowicz B, Henry RR (2015) Sodium-glucose cotransporter inhibitors: effects on renal and intestinal glucose transport: from bench to bedside. *Diabetes Care* 38:2344–2353
- Oh YS (2015) Plant-derived compounds targeting pancreatic beta cells for the treatment of diabetes. *Evid Based Complement Alternat Med* 2015:629863
- Omotuyi OI, Ueda H (2015) Molecular dynamics study-based mechanism of nefiracetam-induced NMDA receptor potentiation. *Comput Biol Chem* 55:14–22
- Omotuyi IO, Elekofehinti OO, Ejelonu OC, Obi FO (2013) Mass spectra analysis of H. sabdarifia L anthocyanidins and their in silico corticosteroid-binding globulin interactions. *Pharmacol OnLine* 1:206–217
- Onkaramurthy M, Veerapur VP, Thippeswamy BS, Reddy TM, Rayappa H, Badami S (2013) Anti-diabetic and anti-cataract effects of *Chromolaena odorata* Linn., in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 145:363–372
- Phuwamongkolwivat P, Hira T, Hara H (2014) A nondigestible saccharide, fructooligosaccharide, increases the promotive effect of a flavonoid, α -glucosyl-isoquercitrin, on glucagon-like peptide 1 (GLP-1) secretion in rat intestine and enteroendocrine cells. *Mol Nutr Food Res* 58:1581–1584
- Raman G, Jayaprakasha GK, Brodbelt J, Cho M, Patil BS (2004) Isolation of structurally similar citrus flavonoids by flash chromatography. *Anal Lett* 37:3005–3016
- Scheen AJ (2003) Pathophysiology of type 2 diabetes. *Acta Clin Belg* 58:335–341
- Scheen AJ, Paquot N (2015) 2015 updated position statement of the management of hyperglycaemia in type 2 diabetes. *Rev Med Suisse* 11(1518):1520
- Schertzer JD, Tamrakar AK, Magalhaes JG, Pereira S, Bilan PJ, Fullerton MD, Liu Z, Steinberg GR, Giacca A, Philpott DJ, Klip A (2011) NOD1 activators link innate immunity to insulin resistance. *Diabetes* 60:2206–2215
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675
- Schwede T, Kopp J, Guex N, Peitsch MC (2003) SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res* 31:3381–3385
- Seeliger D, de Groot BL (2010) Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des* 24:417–422
- Shen H, Shao M, Cho KW, Wang S, Chen Z, Sheng L, Wang T, Liu Y, Rui L (2012) Herbal constituent sequoyitol improves hyperglycemia and glucose intolerance by targeting hepatocytes, adipocytes, and beta-cells. *Am J Physiol Endocrinol Metab* 302:E932–E940
- Shiny A, Regin B, Balachandar V, Gokulakrishnan K, Mohan V, Babu S, Balasubramanyam M (2013) Convergence of innate immunity and insulin resistance as evidenced by increased nucleotide oligomerization domain (NOD) expression and signaling in monocytes from patients with type 2 diabetes. *Cytokine* 64:564–570
- Ver Heul AM, Gakhar L, Piper RC, Subramanian R (2014) Crystal structure of a complex of NOD1 CARD and ubiquitin. *PLoS ONE* 9:e104017
- Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V, Stevens RC (2011) Structure of an agonist-bound human A2A adenosine receptor. *Science* 332:322–327
- Yu OH, Yin H, Azoulay L (2015) The combination of DPP-4 inhibitors versus sulfonylureas with metformin after failure of first-line treatment in the risk for major cardiovascular events and death. *Can J Diabetes* 39:383–389