Antidiabetic activity of *Terminalia sericea* Burch. Ex DC constituents

Nolitha Nkobole[a,c], Peter James Houghton[b], Ahmed Hussein[a] and Namrita. Lall[a*]

[a]*Department of Plant Science, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa*

[b]*Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building 150, Stamford Street, London SE1 9NH UK*

[c]*Human Science Research Council's Centre for Science, Technology and Inovation Indicators (CESTII), Population Health, Health Systems and Innovation (PHHSI).*

namrita.lall@up.ac.za

Diabetes mellitus is an endocrine disorder that affects more than 100 million people worldwide. South African plants namely *Terminalia sericea, Euclea natalensis, Warbugia salutaris, Aloe ferox Artemisia afra, Sclerocarya birrea, Spirostachys africana* and *Psidium guajava* were investigated for their *in vitro* antidiabetic and antioxidant activities. *Terminalia sericea* stem bark extract showed the best results against α-glucosidase and α-amylase enzymes. Bioassay-guided fractionation of an acetone extract of *T. sericea* stem bark led to the isolation of four known compounds namely β-sitosterol (**1**), β-sitosterol-3-acetate (**2**), lupeol (**3**), and stigma-4-ene-3-one (**4**), in addition to two inseparable sets of mixtures of isomers [epicatechin-catechin**; (M1)** and gallocatechin-epigallocatechin**; (M2)**. **1** and **3** showed best inhibitory activity on α-glucosidase (IC_{50}:54.5 µM and 66.48). The bio-evaluation of purified compound's inhibitory activity on α-amylase, showed that lupeol and β-sitosterol exhibited IC_{50} values of 140.72 and 216.02 µM respectively against α-amylase. **2**, **M1**, **3** and **M2** were found to be non-toxic to Vero cells. This study is the first to report α-glucosidase, α-amylase of **M1**, **M2**, **2** and **4** isolated from *T. sericea* which validated the traditional use of the bark of *T. sericea* for diabetes in South Africa.

Keywords: Diabetes mellitus, α-glucosidase, α-amylase, *Terminalia sericea*

Diabetes mellitus (DM) is a chronic endocrine disorder that is characterized by hyperglycemia (high blood glucose levels) that results from defects in insulin secretion, insulin action or both [1-3]. According to the World Health Organization (WHO) more than 176 million people are diabetic with about two thirds of these living in developing countries [1, 4]. The prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to augment to 5.4% by the year 2025 [4]. Increase in inactive lifestyle, consumption of food rich in energy and obesity are some of the contributory factors in escalating number of diabetic patients [1]. There are 2 main types of diabetes that exist namely type-1 and type-2 DM. Type-1 accounts for 5-10% of cases whilst type-2 is responsible for 90-95% cases [5]. Antioxidants have important role in controlling diabetes mellitus. Low levels of plasma antioxidants is a risk factor associated with diabetes [6].

Complications of diabetes include retinopathy; arterosclerosis, kidney failure, etc. which are the leading cause of mortality amongst diabetics. These complications arise as a result of low levels of plasma antioxidants which result in oxidative stress [7]. Plants produce biological active compounds responsible for different activities like antioxidant. In addition plants also produce antioxidants such as ascorbic acid, and tocophenols [8]. It has therefore been suggested that antioxidant action may be an important property of plant medicines associated with diabetes. Medicinal plants have been used since prehistoric times in counties all over the world including South Africa [9]. Majority of people still depends on plants for primary health care.

For α-glucosidase assay acetone extracts of eight plants were tested based on the method as described by Collins et al. (1997). The enzyme, α-glucosidase (EC3.2.1.20) and the substrate were purchased from Sigma Chemical Co., (St Louis, MO, USA). α-Amylase inhibition assay was performed using the chromatogenic method adopted from Sigma-Aldrich according Bernfeld (1955) and modified by Deutschlander et al., (2009). Antioxidant activities of acetone extracts and purified compounds were investigated using the 1, 2-diphenyl-2-picrylhydrazil (DPPH) (Sigma-Aldrich) antioxidant assay according to du Toit *et al*. (2001). The cytotoxicity of crude extract and the isolated compounds of *T. sericea* was investigated by using XTT-based colorimetric assay Cell Proliferation Kit II (Boehringer-Mannheim) following the method as described by Roche (2004). As the extract of *T. sericea* showed good hypoglycemic activity, this extract was further selected for its cytotoxicity evaluation. The final concentration of crude extract tested ranged from 3.125-

400 µg/ml. These values were calculated using Graph Pad Prism 4 programme. The final results are expressed as the mean (standard deviation, ± SE.S). The group means were compared using ANOVA test (MSTATC software, East Lansing, MI, USA) and the Duncan's Multiple range Test was applied to compare the means. Values were determined to be significant when p was less than 0.05 ($p<0.05$).

The results of α-glucosidase and α-amylase inhibitory activities of the plant extracts and compounds isolated from *T. sericea* are shown in table 2. Eight plant extracts were tested for α- glucosidase and α-amylase inhibitory activity. The inhibition percentage of extracts ranged from 47.15 ± 0.02 to 97.57 ± 0.01 at 0.2 mg/ml. Of the tested extracts *T. sericea* and *S birrea,* demonstrated activity on both enzymes ($p<0.05$). Our study correlates with that of [10] where *T. sericea* extract exhibited a considerable α-glucosidase inhibitory activity (IC_{50} value; 92 µg/ml). In the study done by [11], stem bark of a similar species; *T. superba* extracts demonstrated α-glucosidase inhibitory activity and the active constituents were reported to be gallic acid and methyl gallate. On the other hand, the aqueous methanolic extract of *T. chebula* was found to possess potent rat intestinal maltase inhibitory activity. Bioassay guided fractionation of this plant led to the isolation of three active ellagitannins namely chebulanin, chebulagic acid and chebulinic acid which also demonstrated potent intestinal maltase activity [12]
Spondias mombin (Anacardiceae) and its isolated compounds exhibited significant enzyme inhibitory activity against α-amylase ($p<0.05$). This plant belongs to the same family as *S. birrea* which also demonstrated good inhibitory activity on α-amylase (IC_{50}; 100µg/ml) similar to the results obtained in the present study [13]. The isolation of the chromatographic processing of *T. sericea* total extract led to compound **1-4** which were identified as β-sitosterol, β-sitosterol-3-acetate, lupeol, stigma-4-ene-3-one, in addition to catechin-epicatechin mixture (**M1**), and gallocatechin-epigallocatechin mixture (**M2**) (Figure 1). Compounds **1** and **3** showed best inhibitory activity on α-glucosidase exhibiting IC_{50} value at 54.49.± 0.01 µM and 66.48 ± 0.02 µM respectively ($p<0.05$), on α-amylase, compounds of interest were **1** and **3** which exhibited IC_{50} values at 216.02 and 140.72 µM respectively as compared to the positive drug-control acarbose (IC_{50}; 65.25 µM). In another study **3** inhibited α-amylase enzyme [2]. According to [14] stigmast-4-en-3-one, a similar compound to stigma-4-ene-3-one produced significant reduction in blood glucose levels of alloxan-induced diabetic rats [15]. Catechins have been found to play an important role both in α-glucosidase and α-amylase enzymes inhibition. Another study reveals that seven plants tested for α-glucosidase enzyme inhibition and all the extracts exhibited potent inhibition of the enzyme [**17**]. Catechin was amongst the phenolic compounds that were

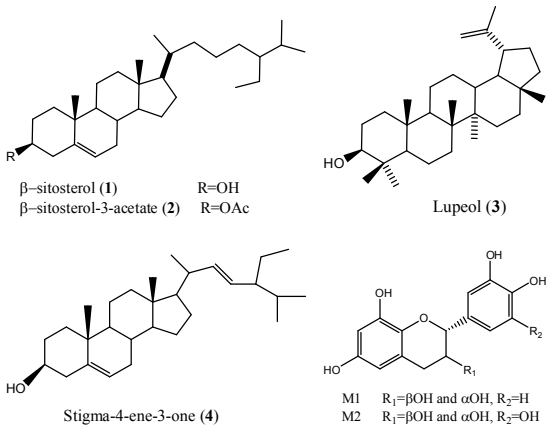

Figure 1: Chemical structure of compounds isolated from the stem bark of *T. sericea*

identified as potential α-glucosidase inhibitors [17**]**. In another study, *in vitro* tests of epigallocatechin gallate, epigallocatechin and epicatechin showed inhibitory activities on α-glucosidase and α-amylase [16].

Table 3 depicts the DPPH scavenging activity of the plant extracts and compounds isolated from *T. sericea*. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. All six tested plant extracts showed good activity ($p<0.05$). *S. birrea, E. natalensis* and *T. sericea* exhibited the highest activity as they showed minimal EC; at 94.78 ± 0.03; 94.35 ± 0.01 and 93.96 ±0.01µg/ml respectively when compared to ascorbic acid (95.80 ± 0.01µg/ml), a well-known antioxidant (Table 2). These plant extracts demonstrated significant results ($p<0.05$). This was followed by *A. afra, P. guajava* and *W. salutaris* (% inhibition= 90.084 ± 0.02; 90.16 ± 0.04 and 70.07 ±0.01µg/ml, table 2). Results of **M1**, **M2** and **3** showed valuable high radical activity exhibiting EC_{50} values of 1.64; 2.69 and 3.66 µM. **M1** and **M2** inhibited DPPH by more than 95% suggesting they had scavenged the whole amount of DPPH [11]. The two isolated isomers namely epigallocatechin-gallocatechin, epicatechin-catechin are polyphenolic plant antioxidants. Catechin and epicatechin are epimers that are abundant in nature. On the other hand, epigallocatechin and gallocatechin contain an additional hydroxyl group when compared to the former [17]. Flavonoids have been reported as being potential therapeutic agents for type 1 diabetes [18]. Therefore, currently there is intensive focus on polyphenolic phytochemicals such as flavonoids [21]. It was previously reported that catechin and epicatechin that were isolated from *Eysenhardtia subcoriacea* demonstrated strong radical scavenging properties against DPPH [19]. Our results are in agreement with the findings of Yu et al. (2007), where epicatechin, isolated from *Garcinia mangostona* was found to exhibit significant antioxidant activity in free radical scavenging test ($p<0.05$).

Table 1. Antidiabetic plants investigated in this study.

Species	Part used	Family	Specimen no	% Yield
Terminalia sericea Burch. Ex DC	Stem bark	Combretaceae	PRU 38564	25
Psidium guajava L	Leaves	Myrtaceae	PRU 54544	30
Sclerocarya birrea (A.Richi.) Hochst. subsp. caffra	Stem bark	Anacardiaceae	PRU 4558/100	15
Warbugia salutaris Bertol.f.) Chiov.	Stem bark	Canellaceae	PRU 094845	14.5
Artemisia afra Jacq. ex Willd.	Leaves	Asteraceae	PRU 112085	15
Aloe ferox Mill	Leaves	Aloaceae	PRU 110308	10
Euclea natalensis A.DC	Root bark	Ebenaceae	PRU 095059	15
Spirostachys africana Sond	Leaves	Euphorbiaceae	PRU 8434	10

Table 2. Inhibitory and antioxidant activities of different plant extracts on α-glucosidase, α-amylase enzymes and DPPH at 0.2 mg/ml

Plant	α-glucosidase Inhibition % (SD)	α-amylase Inhibition % (SD)	DPPH Inhibition % (SD)
T. sericea	97.44 ± 0.03*	91.91 ± 0.10*	93.96 ±0.01*
S. birrea	97.44 ± 0.04*	94.94 ± 0.01*	94.78±0.03*
A. *afra*	47.15 ± 0.02	74.00 ± 0.01*	90.084±0.02*
E. natalensis	92.60 ± 0.04*	74.54 ± 0.04*	94.35 ±0.01*
P. gaujava	62.74 ± 0.19*	89.14 ± 0.01*	90.16 ±0.04*
W. salutaris	71.84 ± 0.27*	89.21 ± 0.06*	70.07 0.01*
A. ferox	NI[a]	NI[b]	NT
S. africana	NI[a]	NI[b]	NT
Acarbose	80.63 ± 0.03*	73.40 ±0.03*	-
Ascobic acid (Vit C)	-	-	95.80 ± 0.01*

Values are means ± SD of three concentrations.
* $p<0.05$.
NI [a, b]: no inhibition
NT: not tested

Table 3. Inhibitory activities of compounds isolated from *T. sericea* on α-glucosidase, α-amylase, DPPH and Vero cell lines

Compounds	IC_{50} ά –Glucosi (μM)	IC_{50} ά –Amylas (μM)	EC_{50} DPPH (μM)	IC_{50} Vero Cell lines (μM)
Acarbose (positive dru Control)[b]	93.22*	60.25*	a	a
Vitamin C[c]	a	a	2.52*	
Doxurubicin[d]				0.41
β-sitosterol (1)	54.49*	215.95*	N/A	197.72*
β-sitosterol-acetate (2)	129.36*	N/A	N/A	482.25
stigma-4-ene-3-one (3)	164.87*	N/A	N/A	N/A
Epigallocatechin & Gallocatechin (MI)	119.34*	328.06*	1.64*	653.02*
Epicatechin & Catechi	255.796*	304.89*	2.69*	689.00*
Lupeol (4)	66.48	140.72*	3.66*	705.14*

Values are means ± SD of three concentrations.
* $p<0.05$.
[a] Not tested
[b] Positive drug control for α-glucosidase and α-amylase
[c] Positive drug control for DPPH
[d] Positive drug control for cancer

Cytotoxicity evaluation of the plant extract and compounds is reported table 3. *T. sericea* acetone extract and the isolated compounds did not show toxicity against VK cells. Catechin derivatives: **M1** and **M2** did not demonstrate any toxicity on Vero cell lines. This confirms the findings by [20] where *Erythroxylum cuneatum* extract was tested on Vero cells, demonstrating no toxicity (IC_{50} value of 366 μg/ml). The active compound isolated from the plant was (+)-catechin [20]. The crude extract of *T. sericea* as well as compounds showed *invitro* antidiabetic activity on all assays. Compounds were also tested for cytotoxicity on Vero cell lines. This study is the first to report α-glucosidase, α-amylase and antioxidant properties of **MI**, **M2**, **2** and **4** isolated from *T. sericea*. In addition, **MI**, **M2**, **2** and **4** are isolated from *T. sericea* for the first time. The extract of *T. sericea* is moderately toxic to Vero cells. Ideally water extracts (which are less toxic) are used traditionally however due to their low activity other organic solvents are recommended for *in vitro* studies.

Experimental

T. sericea was chosen for the isolation and identification of pure compounds. Air-dried powdered stem bark of *T. sericea* (1.8 kg) was extracted with 100% acetone for 48 hours. The acetone extract was filtered and evaporated under reduced pressure. The total concentrated extract (80 g) was subjected to silica gel column chromatography (CC, size 10 x 30 cm) using hexane/ethyl acetate mixtures of increasing polarity (0-100%) followed by 100% methanol (MeOH), a total 30 fractions (250 ml) were collected. The fractions were combined on the basis of thin layer chromatography (TLC) analysis which lead to 13 main fractions. The 13 main fractions were tested on α-glucosidase for their inhibitory activity. Main fraction **1** was subjected to silica gel CC and eluted using hexane/ ethyl acetate mixture which yielded compound **1** and **5** sub-fractions (a-f). Sub-fraction a was rechromatographed on silica gel column which was eluted with ethyl acetate-hexane to give compounds **2** and **3**. Main fraction 3 was subjected to sephadex CC and eluted with 98% ethanol which yielded compound **4**. Main fraction 6 was further separated on silica gel CC using hexane/ethyl acetate mixtures of increasing polarity to give isomer mixtures MI and M2. The structural elucidation of isolated compounds (figure 1) were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H and ^{13}C NMR) data.

References

[1] Abo KA, Fred-Jaiyesimi F, Jaiyesimi AE. **2008** Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *Journal of Ethnopharmacology*, ***115***, 67-71.

[2] Ali H, Houghton PJ, Soumyanath. **2006** α-Inhibitory activity of some Malaysian plants used to treat diabetes; with reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, ***107***, 449-455.

[3] Coskun O, Kanter M, Korkmaz A, Oter S. **2005** Quercetin, a flavonoid antioxidant, prevents streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. *Pharmacological Research*, ***51***, 117-23.

[4] King H, Aubert RE, Herman WH. **1998** Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* , ***21***, 1414-1431.

[5] Li W, Zhen HC, Bukuru J, Kimpe ND. **2004** Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of Ethnopharmacology*, ***92***, 1-21.

[6] Mccune LM, Johns T. **2002** Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by Indigenous People of the North American boreal forest. *Journal of Ethnopharmacology*, ***82***, 197-205.

[7] Muaka MM, Longo-Mbenza B, Mona DT, Okwe AN. **2010** Reduced risk of metabolic syndrome due to regular intake of vegetables rich in antioxidants among African type 2 diabetics. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, ***4***, 132–136.

[8] Larson R.A. **1988** The antioxidants of higher plants. *Phytochemistry*, ***27***, 969-9783

[9] Van Wyk BE, Wink M. **2004** Medicinal Plants of the World: An illustrated scientific guide to important medicinal plants. Briza Publication, Pretoria.

[10] Tshikalange TE, Meyer JJM, Lall N, Munoz E, Sancho R, Van Der Venter M, Oosthuizen V. **2008** *In vitro* anti-HIV-1 properties of ethnobotanically selected South African plants used in the treatment of sexually transmitted diseases. *Journal of Ethnopharmacology,* ***119***, 478-481.

[11] Wansi JD, Lallemand M, Chiozem DD, Toze F.A. Mbaze LM, Naharkhan S, Iqbal MC, Tillequin F, Wandji J, Fomum ZT. **2007** α-Glucosidase inhibitory constituents from stem bark of *Terminalia superba* (Combretaceae). *Phytochemistry*, ***68***, 2096–2100.

[12] Gao H, Houng YN, Xu PY, Kuwabata J. **2007** Inhibitory effect on alpha glucosidase by the fruits of *Terminalia chebula* Retz. *Food Chemistry*, ***105***, 628-634.

[13] Adediwura FJ, Kio A, Richardo W. **2008** α-Amylase inhibitory effect of 3-β-olean-12-en-3-y (9Z) hexadec-9-enoate isolated from *Spondias mumbin* leaf. *Food Chemistry*, ***116***,285-288.

[14] Jamaluddin F, Mohamed S, Lajis N. **1995** Hypoglycaemic effect of Stigmast-4-en-one, from *Parkia speciosa* empty pods. *Food Chemistry*, ***54,*** 9-13.

[15] Bayners JW. **1991** Role of oxidative stress in development of complications in diabetes. *Diabetes, **40***, 405-412

[16] Chunqing S, Xiaoping Y. Rengang M. **2009** Method of isolation and purification of main catechin from tea polyphenols and its glycosidase activity. CN 101492440 A 20090729.

[17] Li T, Liu J, Zhang X, Ji G. **2007** Antidiabetic activity of lipophilic (-)-epigallocatechin-3-gallate derived under its role of α-glucosidase inhibition. *Biomedicine and Pharmacotherapy, **61***, 91-96.

[18] Yazdanparast R, Ardestani A, Jamshidi SH. **2007** Experimental diabetes treated with *Achillea santolina*: effect on pancreative oxidative parameters. *Journal of Ethnopharmacology, **112***, 13-18.

[19] Narvaez-Mastache J, Novillo F, Delgad G. **2008**. Antioxidant aryl-prenylcoumarin, flavan-3-ols and flavonoids from *subcoriacea*. *Phytochemistry*, ***69***, 451-456.

[20] Prayong P, Barusrux S, Weerapreeyakul, N. **2008** Cytotoxicity activity screening of some indigenous Thai plants. *Fitoterapia, **79***, 59-601.

[21] Yu L, Zhao M, Yang B, Zhao Q, Jiang Y. **2007** Phenols from hull of *Garcinia mangostona* fruit and their antioxidant activities. *Food Chemistry, **104***, 176-181.

[22] Collins RA, Ng TB, Fong WP, Wan CC Yeung HW. **1997** A comparison of human immunodeficiency virus type 1 inhibition by partially purified aqueous extracts of Chinese medicinal herbs, *Life Sciences, **60***,345–351.

[23] du Toit R, Volsteedt Y, Apostilides Z. **2001**. Comparison of antioxidant content of fruits, vegetables and teas measured as Vitamin C equivalents. *Toxicology, **186***, 83-89.

[24] Deutschländer MS, van de Venter M, Roux S, Louw J, Lall N. **2009** Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of Ethnopharmacology*, **124**, 619-24