



Antidiabetic activity of pentacyclic triterpenes and flavonoids
isolated from stem bark of *Terminalia sericea* Burch.Ex DC

BY

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree of Masters of science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed;

Date:.....

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LIST OF ABBREVIATIONS

AGE:	Advanced glycosylated end products
DM:	Diabetes mellitus
DMSO:	Dimethylsulfoxide
DPPH:	1, 2-diphenyl-2-picrylhydrazil
ERK:	Extracellular signal-regulated kinases
GLUT4:	Glucose transporter
IDDM:	Insulin dependent diabetes mellitus
IRS-1:	Insulin receptor substrate 1
MAPK:	Mitogen-activated protein (MAP) kinases
NIDDM:	Non-insulin dependent diabetes mellitus
NMR:	Nuclear Magnetic Resonance
PEPCK:	Phosphoenopyruvate carboxykinase
TLC:	Thin Layer Chromatography
T1DM:	Type -1 diabetes mellitus
T2DM:	Type- 2 diabetes mellitus
UV:	Ultra violet
WHO:	World Health Organization
XTT:	2, 3-bis-[2-methoxy-4-nitrophenyl]-2H-tetrazolium-5-carboxanilide

Antidiabetic activity of pentacyclic triterpenes and flavonoids isolated from the stem bark of *Terminalia sericea* Burch. Ex DC

Abstract

Diabetes mellitus (DM) represents a series of metabolic conditions associated with hyperglycemia and caused by defects in insulin secretion, and/ insulin action. Exposure to chronic hyperglycemia may result in microvascular complications in the retina, kidney or peripheral nerves. According to the World Health Organization (WHO) global burden of disease, more than 176 million people are diabetic with about two thirds of these living in developing countries. With a long course and serious complications that often result in high incidences of mobility and mortality rate, the treatment of diabetes is often costly. The management of this disease is not without side effects and this is a challenge to the medical system. This has led the researches to seek new antidiabetic agents from plants.

Acetone extract of 8 plants namely *Terminalia sericea* Burch. Ex DC, *Euclea natalensis* A.DC, *Warbugia salutaris* Bertol.f.) Chiov., *Artemisia afra* Jacq.ex Willd., *Aloe ferox* Mill, *Sclerocarya birrea* (A.Richi.) Hochst. subsp. *caffra* , *Spirostachys Africana* Sond and *Psidium guajava* L were evaluated for antidiabetic and antioxidant properties. In addition extracts were tested for cytotoxicity. Different parts of all these plants are traditionally used in South Africa for diabetes treatment. Plants were selected based on ethnobotanical information and phytochemical constituents. For determining inhibitory activity against each enzyme (α -glucosidase and α -amylase), all extracts were tested at concentration that ranged from 2×10^{-5} to 0.2mg/ml for α -glucosidase and 0.025 to 1.25mg/ml for α -amylase and fifty percent inhibition or higher was taken

as significant ($p < 0.05$). The extracts of *A. ferox* and *S. africana* showed no inhibition against α -glucosidase at the highest concentration tested (0.2mg/ml) whereas *A. afra* showed weak inhibition (47.15%). *T. sericea* showed to be a potent inhibitor of α -glucosidase exhibiting 97.44 % inhibition of the enzyme ($p < 0.05$). *W. salutaris*, *S. birrea* and *E. natalensis* also showed good activity on α -glucosidase as they demonstrated 71.84; 97.44 and 92.60 % inhibition respectively ($p < 0.05$). Other plant extracts such as *A. ferox* and *S. africana* did not exhibit any activity on α -glucosidase.

T. sericea and *S. birrea* showed the best inhibitory activity on α -amylase enzyme, exhibiting 91.91 and 94.94 % inhibition respectively at 1.25mg/ml. *A. afra*, *E. natalensis*, *P. guajava* and *W. salutaris* also showed good inhibitory activity on α -amylase enzyme at 1.25mg/ml which was the highest concentration tested ($p < 0.05$).

Low levels of plasma antioxidants is a risk factor associated with diabetes therefore, it has been suggested that plant-based medicines that contain antioxidant properties add an advantage in curbing complications that arise during DM aetiology. The antioxidant activity of plant extracts was carried out using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay. Six plant extracts which showed good α -glucosidase and α -amylase inhibitory activity were evaluated for antioxidant activity. The radical scavenging activity was measured in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance (EC_{50}). The EC_{50} is the amount of antioxidants necessary to decrease initial DPPH absorbance by 50%. All 6 tested plant extracts showed good activity. *W. salutaris* and *T. sericea* demonstrated the highest activity exhibiting EC_{50} values of 5.08 and 5.56 μ g/ml respectively as compared to ascorbic acid/Vitamin C (EC_{50} =2.52 μ g/ml), a well-known potent antioxidant. This was followed by *P. guajava* (EC_{50} =6.97 μ g/ml); *E. natalensis* (EC_{50} =8.46 μ g/ml) and *S. birrea* (EC_{50} =9.41 μ g/ml). *A. ferox* showed EC_{50} value of 48.53 μ g/ml.

It has been suggested that plant extracts and compounds must undergo toxicity test for safety before drug discovery is taken into consideration. Due to the large number of plants screened in this study and limited resources in our laboratory, only the acetone extract of *T. sericea* (which demonstrated good α -glucosidase and α -amylase inhibitory activities) was tested for cytotoxicity. Acetone extract of *T. sericea* demonstrated moderate toxicity against primary vervet monkey kidney cells (VK) cells exhibiting IC_{50} values of 20.94 $\mu\text{g/ml}$ when tested at 400 $\mu\text{g/ml}$. Consequently, the acetone extract of *T. sericea* was selected for the isolation and identification of bioactive compounds. A bio-assay guided fractionation of the acetone extract of *T. sericea* led to the isolation of 4 pure compounds namely β -sitosterol, β -sitosterol-3-acetate, lupeol and 3-one-stigmasterol and two sets of mixtures of isomers (epicatechin-catechin; MI1 and epigallocatechin-gallocatechin; MI2).

Antidiabetic, antioxidant and cytotoxicity activities of isolated compounds were evaluated. β – Sitosterol and lupeol showed best inhibitory activity on α -glucosidase exhibiting 50% inhibitory concentration (IC_{50}) value of 54.50 μM and 66.48 μM respectively ($p < 0.05$). This was followed by the MI2; epigallocatechin-gallocatechin ($IC_{50}=119.34 \mu\text{M}$); β -sitosterol-3-acetate ($IC_{50}=129.34 \mu\text{M}$); 3-one-stigmasterol ($IC_{50}=164.87 \mu\text{M}$) and the MI1; epicatechin-catechin ($IC_{50}=255.76 \mu\text{M}$). During the evaluation of purified compound's inhibitory activity on α -amylase, compounds of interest were lupeol and β -sitosterol which exhibited IC_{50} values of 140.72 μM and 216.02 μM respectively as compared to the positive drug-control acarbose ($IC_{50}=65.25 \mu\text{M}$). Epicatechin-catechin and epigallocatechin-gallocatechin also demonstrated α -amylase inhibitory properties and the IC_{50} values were found to be lower than 100 $\mu\text{g/ml}$. Epigallocatechin-gallocatechin, epicatechin-catechin and lupeol showed good free radical scavenging activity as they inhibited DPPH by 98.19; 96.98 and 70.90 % at 100 $\mu\text{g/ml}$ respectively ($p < 0.05$). The DPPH scavenging activity was very low in case of 3-one-stigmasterol (21.5% inhibition), whilst β -sitosterol and its derivative β -sitosterol-3-acetate did not show any activity.

During cytotoxicity evaluation of pure compounds against monkey kidney cells, all the compounds except β -sitosterol did not inhibit the growth of these cells lines at the highest concentration tested (200 μ g/ml). β -Sitosterol showed moderate toxicity exhibiting IC_{50} values of 197.72 μ M. β -Sitosterol-3-acetate, epicatechin-catechin, lupeol and epigallocatechin-gallocatechin were found to be non-toxic to Vero cells as 100% cell viability was observed when Vero cells were exposed to these samples at 200 μ g/ml.

The compounds isolated and the extract of *T. sericea* demonstrated significant antidiabetic and antioxidant properties as compared to well known drugs acarbose (a known α -glucosidase and α -amylase inhibitor) and Vitamin C (a well known antioxidant). This study is the first to report α -glucosidase, α -amylase and antioxidant properties of epicatechin-catechin, epigallocatechin-gallocatechin, β -sitosterol-3-acetate and stigma-4-ene-3-one isolated from *T. sericea*. In addition, epicatechin-catechin, epigallocatechin-gallocatechin, β -sitosterol-3-acetate and stigma-4-ene-3-one are isolated from *T. sericea* for the first time. Overall all results scientifically validated the traditional use of the bark of *T. sericea* for diabetes in South Africa.