Isolation and identification of a novel anti-diabetic compound from *Euclea undulata* Thunb.

by

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Declaration

I declare that this thesis, which I hereby submit for the degree PhD Plant 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.

Signature: **Date:**

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Abstract

Four plant species traditionally used for the treatment of diabetes by South African traditional healers and herbalists were investigated for hypoglycaemic activity. Species included *Schkuhria pinnata* (Lam.) Cabrera, *Pteronia divaricata* (P.J. Bergius) Less *Elaeodendron transvaalense* (Burtt Davy) R.H. Archer and *Euclea undulata* Thunb var. *myrtina* (Burch.) Hiern.

Acetone and ethanol plant extracts were prepared and tested *in vitro,* for glucose utilization, at concentrations of 12.5 µg/ml on three cell lines namely; Murine C2C12 myocytes, Chang liver cells and 3T3-L1 preadipocytes. Metformin, at a concentration of 1 µM (0.166 µg/ml) was used as positive control for hepatic cells and insulin at a concentration 1 µM (5.7 µg/ml) for myocytes and preadipocytes. Toxicity tests were done for all extracts on preadipocytes and hepatic cells, but not on myocytes as these cells were exposed to the extract for only a short period (1 hour) during the hypoglycaemic bioassay. Preadipocytes and hepatic cells were exposed to the plant extracts for 48 hours. The four plant extracts were further investigated for hypoglycaemic activity by evaluating inhibiting effects on carbohydrate-hydrolising enzymes alpha-glycosidase and alpha-amylase.

In vitro hypoglycaemic analysis revealed that acetone and ethanol plant extracts of *S. pinnata*, *E. undulata* and *E. transvaalense*, displayed hypoglycaemic activity in one or more of the various cell lines, whereas, *P. divaricata* showed no hypoglycaemic activity. The best results were obtained with the ethanol and acetone extracts of *S. pinnata* in

preadipocytes with a glucose uptake of 148.2% and 79.6% respectively, above control (100%). However, about 50% preadipocytes survived on exposure to the extracts of *S. pinnata* at 12.5 µg/ml indicating significant cytotoxicity. Glucose uptake of 63.3% was observed by the ethanol extract of *S. pinnata* on hepatic cells. *E. transvaalense* showed hypoglycaemic activity on preadipocytes exhibiting glucose uptake of 38.6% above control 100%. Glucose uptake of 62.2 % were obtained by the *E. undulata* extract in C2C12 myocytes, with 100% cell viability. *E. undulata* scored a +3 and was chosen for further analysis. Antidiabetic activity and toxicity of the plant extracts were taken into consideration when scoring was applied.

Alpha-glucosidase and alpha-amylase results indicated that *P. divaricata* extract inhibited alpha-glucosidase (IC_{50} 31.22 µg/ml) whereas *E. undulata* (IC_{50} 2.80 µg/ml) and *E. transvaalense* (IC_{50} 1.12 µg/ml) extracts inhibited alpha-amylase. Results obtained indicated that all four plant extracts tested have the ability to lower blood glucose levels to some extent and in different manners and therefore corroborate the ethnomedicinal use of these four species in the treatment of diabetes.

Phytochemical studies of a crude acetone extract of the root bark of *E. undulata* var. *myrtina* produced a new α-amyrin-3O-ß-(5-hydroxy) ferulic acid compound (**1**), and three known compounds; betulin (**2**), lupeol (**3**) and epicatechin (**4**). The chemical structures were determined by spectroscopic means. *In vitro* assays on C2C12 myocytes revealed that (**2**) (21.4%) and (**4**) (166.3%) were active in lowering blood glucose levels whereas (**1**) (IC_{50} 4.79 µg/ml) and to a lesser extent (**4**) (IC_{50} 5.86 µg/ml) and (**3**) (IC_{50} 6.27 µg/ml) inhibited alpha-glucosidase. These results indicated that the crude, *E. undulata* acetone extract does contain compounds that display hypoglycaemic activity.

The hypoglycaemic activity of four plant species including *E. undulata,* and the four isolated purified compounds, are reported for the first time.

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