The phytochemical investigation and antidiabetic activity of *Cissampelos capensis* and *Strychnos henningsii* the Eastern Cape medicinal plants

Nehemiah Latolla and Buyiswa Hlangothi

Department of Chemistry, Faculty of Science, Nelson Mandela University, Gqeberha, South Africa, 6019

Introduction

- Diabetes mellitus is recorded as a significant health crisis in South Africa.[1]
- The Eastern Cape Province is estimated to have the highest population living in poverty and rural areas.[2, 3]
- Where the scourge of public health care inadequacies, socioeconomic issues, cultural - and traditional beliefs, have rendered residents reliant on various medicinal plants for the management of diabetes. [4, 5–7]
- *Cissampelos capensis* L.f. and *Strychnos henningsii* Gilg. known for their rich diversity of isoquinoline and indole alkaloids, respectively, are utilised by locals in the management of diabetes.[2,8,9]
- Further phytochemical groups associated with their antidiabetic activity are; phenols, flavonoids, and terpenes.[2]
- Thus, this study focused on the phytochemical investigation and antidiabetic activity of *C. capensis* and *S. henningsii*, from the Eastern Cape, in the quest for novel compounds for the management of diabetes.

Figure 1: *Cissampelos capensis* L.f. (left) and *Strychnos henningsii* Gilg. (right)

Methodological Overview

To determine the phytochemical profile by employing HPTLC and quantitative UV-spectrometry.

To identify the known alkaloidal chemical profile through analytical LCMS methods.

To screen the crude extracts and isolated compounds for their antidiabetic activity through *in vitro* biological assays.

To fractionate, isolate, and characterise pure compounds using various spectroscopic techniques.

Results and Discussion

- Qualitative HPTLC detected the presence of alkaloids and phenols in both methanolic and total tertiary alkaloidal extract (TTA), while flavonoids, terpenes and triterpenes were only detected in the methanolic extracts. The HPTLC profiling of *Strychnos henningsii* is shown in **Figure 2**.
- Quantitative UV-Vis Spectrophotometry was employed to estimate total phytochemical content and the results for the various extracts of *Cissampelos capensis* are depicted in **Figure 2**.
- The total alkaloid content was estimated the highest, followed by the flavonoid, phenolic, and terpene content for both plant extracts. However, only alkaloid and phenolic content was detected in TTA extracts.

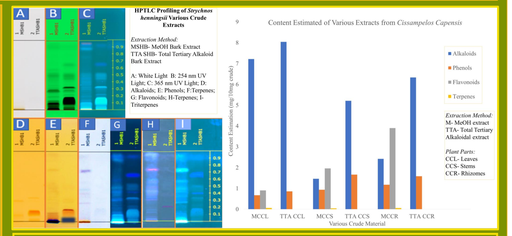

Figure 2: HPTLC profiling of *S. henningsii* (left) and phytochemical content estimation of *C. capensis* (right)

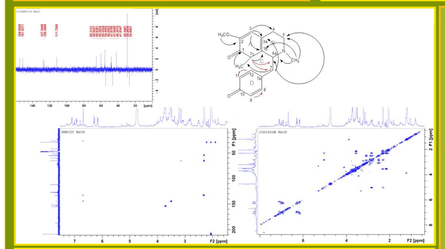

Figure 3: DEPT, HMBC, and COSY NMR of compound CC-4 from *C. capensis*

- Fractionation and purifications of *C. capensis* crudes resulted in the isolation of five proaporphine alkaloids: glaziovine **(CC-1)**, pronuciferine **(CC-2),** cissamaline **(CC-3),** cissamanine **(CC-4),** and cissamidine **(CC-5)**, as depicted in **Figure 4**.
- The latter three proaporphine alkaloids were novel while the former two are isolated from the stems for the first time.
- Various spectroscopic techniques were use in the elucidation of the compounds and the DEPT, HMBC, and COSY NMR of cissamanine is shared in **Figure 3**.

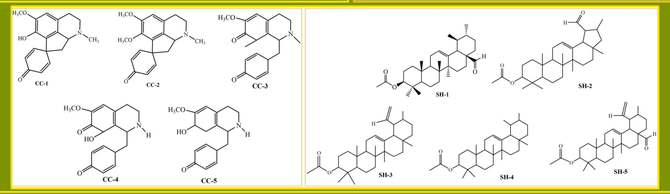

Figure 4: The proaporphine alkaloids CC-1 to CC-5 from *C. capensis* and pentacyclic triterpenes SH-1 to SH-5 from *S. henningsii*

- Exploration of *S. henningsii* lead to the isolation of five novel pentacyclic triterpenes: henningsal **(SH-1)**, henninglupal **(SH-2),** henningcyl **(SH-3),** 19-ethylene henningcyl **(SH-4),** and 19-ethylene henningsal **(SH-5)**, as shown in **Figure 4**.
- The various crude extracts of both plants exhibited concentration dependant cytotoxicity against Caco-2 cells, where TTA extracts exhibited higher cytotoxicity.

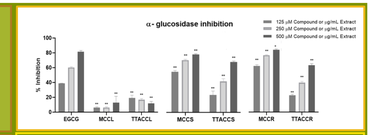

Figure 5: The α-glucosidase inhibition of the *C. capensis* crude extracts. Data points represent the Mean ± SD (n = 3). *P < 0.05; **P < 0.005.)

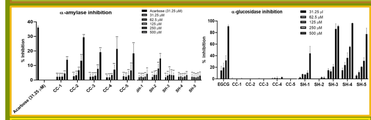

Figure 6: The α-amylase – and α-glucosidase inhibition of pure compounds CC-1 to CC-5 and SH-1 to SH6. Data points represent the Mean ± SD (n = 3). *P < 0.05; **P < 0.005.)

- Crude extracts from both plants exhibited high antioxidant activity (ORAC and DPPH assay), generally the TTA extracts showed lower antioxidants.
- α-Glucosidase inhibition of the various crudes showed promising inhibition, where the MCCS and MCCR extracts showed inhibition exceeding the standard used (**Figure 5**).
- All isolated compounds exhibited concentration dependant cytotoxicity and showed cytotoxicity comparable to the of the standard (melphalan) at 62.5 µM.
- All the pentacyclic triterpenes showed promising α-amylase – and α-glucosidase inhibition, while the proaporphine alkaloids only exhibited α-amylase inhibition **(Figure 6).**

Conclusions

- The phytochemical investigation supports the presence of alkaloids, phenols, flavonoids, and terpenes. Which have been associated to the antidiabetic activity of *C. capensis* and *S. henningsii.*
- Five proaporphine alkaloids (three novel) and five novel pentacyclic triterpenes were isolated from *C. capensis* and *S. henningsii* respectively.
- The crude extracts and isolated compounds exhibited concentration dependent cytotoxicity, high antioxidant activity, and promising alpha-glucosidase and/or alpha-amylase inhibition, respectively.
- The study contributes new knowledge as it validates the use of these plants by locals to manage diabetes.
- And offering potential lead compounds for diabetes type II through the isolation of the novel compounds.

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