Botanical biopesticides research and development: the potential of *Guiera senegalensis*

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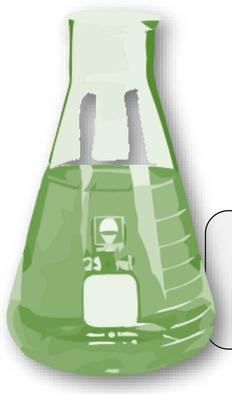
Introduction

Plants produce a vast array of secondary metabolites, which are long known for playing vital roles in resistance and tolerance to abiotic and biotic stress agents. There are several anthropological evidences on the use of plants and plant-based formulations to protect crops and humans against the harmful effects promoted by insects that date back to more than 3000 years ago. Considering the well-known environmental concerns associated with the widespread use of synthetic insecticides, bioprospecting plant extracts with potential insecticidal activity became an active scientific field. Thus, in the present work a methanol extract of leaves from *Guiera senegalensis* J. F. Gmel., a medicinal species commonly distributed in African countries, was characterized and its potential as bioinsecticide assessed by *in vitro* assays.

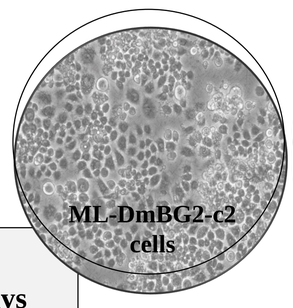
Experimental Design



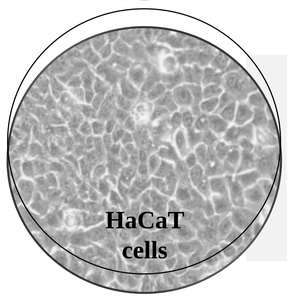
Guiera senegalensis leaves **methanol** extract

Phenolic profile obtained by **HPLC-DAD-ESI/MS** analysis

Cell assays

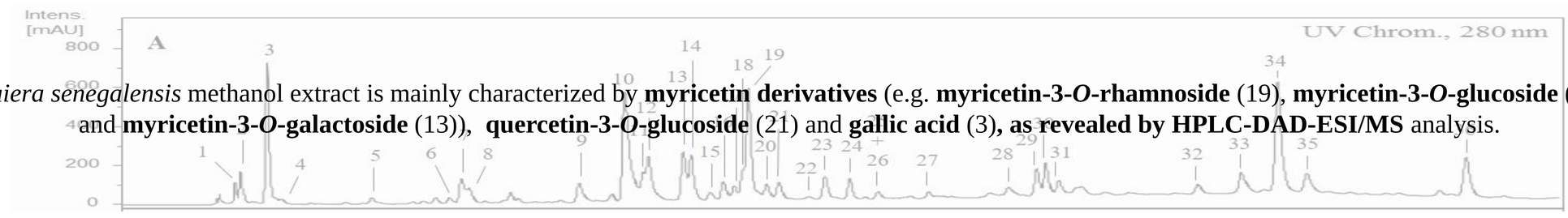


- **Cytotoxicity profile** on **neuronal *Drosophila melanogaster*** and **human cells** – Selective toxicity towards insect cells
- **Cell death mechanisms** on **neuronal insect cells** – mitochondrial and plasma membrane related effects



- **Cytotoxicity** and **photo-safety** profiles on human **keratinocytes**

Results

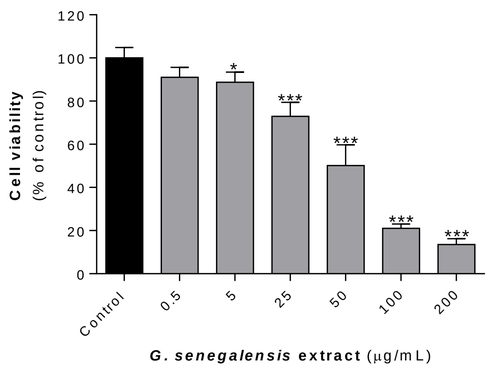


Guiera senegalensis methanol extract is mainly characterized by **myricetin derivatives** (e.g. **myricetin-3-*O*-rhamnoside** (19), **myricetin-3-*O*-glucoside** (14) and **myricetin-3-*O*-galactoside** (13)), **quercetin-3-*O*-glucoside** (21) and **gallic acid** (3)**, as revealed by HPLC-DAD-ESI/MS** analysis.

Cytotoxicity profiles on *Drosophila melanogaster* and human neuronal cells

Guiera senegalensis extract exhibits **selective toxicity for insect cells**

Insect neuronal ML-DmBG2-c2 cell line



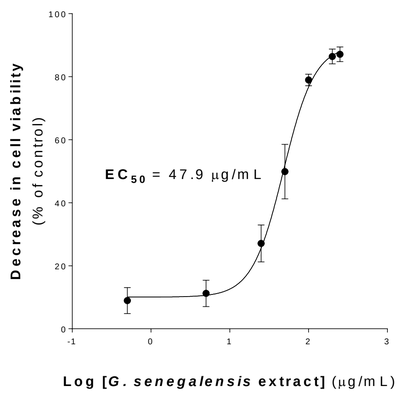


Figure 1. Effect of increasing concentrations of *G. senegalensis* leaves extract on the viability of *Drosophila melanogaster* neuronal cells (ML-DmBG2-c2 cells) evaluated by the MTT reduction assay after a 48 h incubation period. Results are expressed as percentage of control (cells treated with vehicle) and presented as mean ± SD of five independent assays, each one performed in triplicate. * $p \leq 0.05$ *** $p \leq 0.001$.

Human neuronal SH-SY5Y cell line

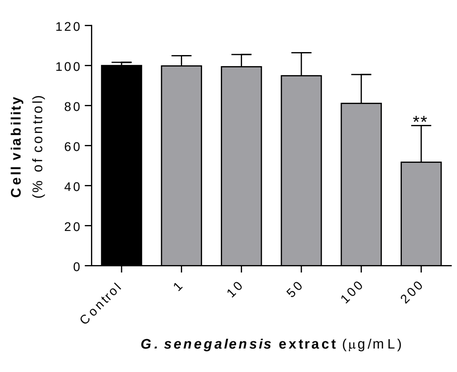
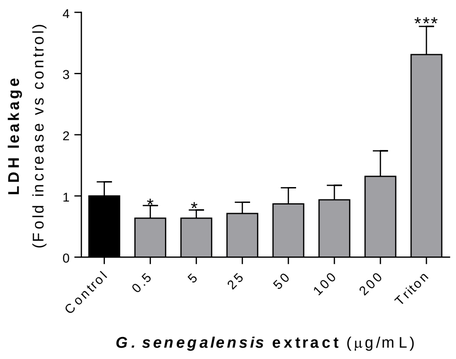
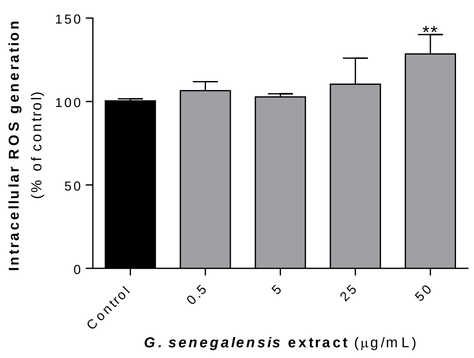


Figure 2. Effect of increasing concentrations of *G. senegalensis* leaves extract on the viability of a human neuronal cell line (SH-SY5Y) evaluated by the MTT reduction assay after a 48 h incubation period. Results are expressed as percentage of control (cells treated with vehicle) and presented as mean ± SD of three independent assays, respectively, each one performed in triplicate. ** $p \leq 0.01$.

The **toxicity** for neuronal insect cells **is mainly related with mitochondrial dysfunctions**





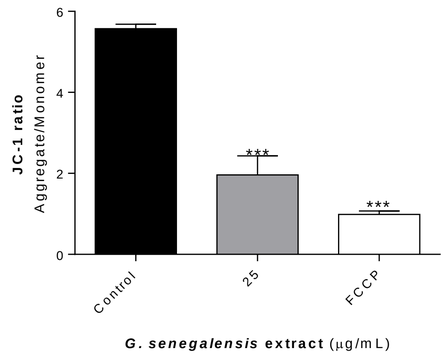


Figure 3. Effect of *G. senegalensis* leaves extract on the membrane integrity, intracellular reactive oxygen species generation and mitochondrial membrane potential of *Drosophila melanogaster* neuronal cells (ML-DmBG2-c2 cells) evaluated by the LDH leakage assay, DCFH-DA probe and the dual-emission potential-sensitive JC-1 probe, respectively, after a 48 h incubation period; Results are expressed as percentage of control (cells treated with vehicle) and presented as mean ± SD of four (LDH leakage and ROS generation) and three independent assays, respectively, each one performed in triplicate. * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$.

Photo-safety profile

Guiera senegalensis extract protects human keratinocytes against the harmful effects of UV radiation

Human keratinocyte HaCaT cell line

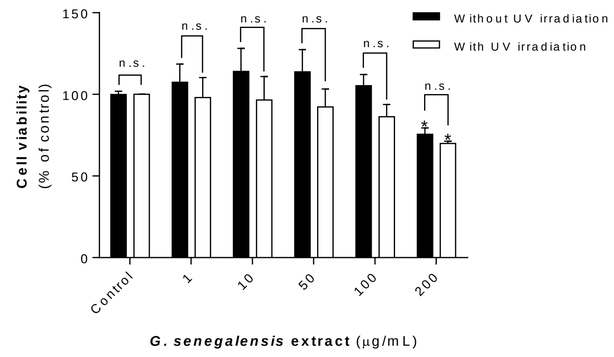


Figure 4. Effect of increasing concentrations of *G. senegalensis* leaves extract on the viability of a human keratinocyte cell line (HaCaT) evaluated by the MTT reduction assay after a 48 h incubation period. Results are expressed as percentage of control (cells treated with vehicle) and presented as mean ± SD of three independent assays, respectively, each one performed in triplicate. * $p \leq 0.05$; n.s. without significant differences.

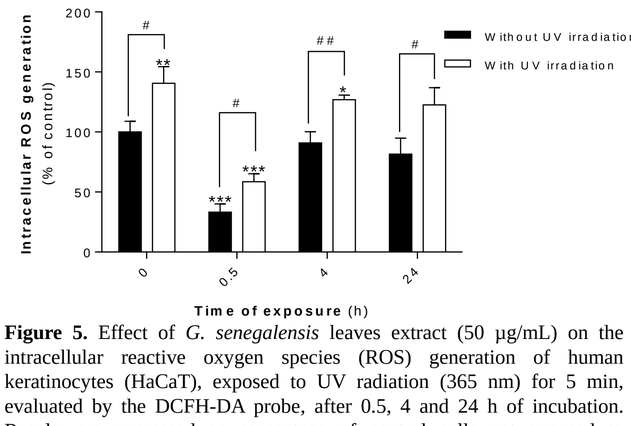


Figure 5. Effect of *G. senegalensis* leaves extract (50 µg/mL) on the intracellular reactive oxygen species (ROS) generation of human keratinocytes (HaCaT), exposed to UV radiation (365 nm) for 5 min, evaluated by the DCFH-DA probe, after 0.5, 4 and 24 h of incubation. Results are expressed as percentage of control cells not exposed to radiation and presented as mean ± SD of three independent assays. *, **, *** Significantly different from control (untreated cells), with $p \leq 0.05$, $p < 0.01$ and $p < 0.001$, respectively. #, ## Significantly different from the cells exposed to UV radiation, with $p \leq 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Conclusions

G. senegalensis leaves extract exhibits a rich profile in phenolic compounds, being particularly rich in flavonol 3-*O*-glycosylated derivatives, such as myricetin-3-*O*-rhamnoside, myricetin-3-*O*-glucoside, myricetin-3-*O*-galactoside and quercetin-3-*O*-glucoside. The extract shows selective cytotoxicity towards a *Drosophila melanogaster* neuronal cell line, while showing no statistically significant decreases on the cell viability of human skin and neuronal cell lines until the concentration of 100 µg/mL. The results on the LDH leakage assay indicate that the reduction of cell viability detected on the MTT assay should not be linked with plasma membrane permeabilization and, consequently, that the observed cytotoxicity does not involve necrosis. Furthermore, the detected loss of mitochondrial membrane potential is a common feature in apoptotic events. The phenolic extract also discloses an interesting ability to protect human keratinocytes against the harmful effects of UV radiation, which may be of relevance considering its potential as a bioinsecticide and the topical use of insecticidal formulations.

Aknowledgements

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