Chapter VII - Conclusions

7.1. Concluding remarks

Regarding the phytochemical study:

This project constitutes the first report on the chemical composition of *C. tinctoria* flowering tops being commercialized in Portugal.

A chemical profile of the bioactive extracts, infusion and AcOEt fraction, was determined yielding a total of sixteen phenolic compounds, being flavonoids the major structural types of compounds present in Portuguese *C. tinctoria* samples.

Three compounds, marein (78), okanin (79) and flavanokanin (88) were successfully isolated using chromatographic techniques and were successfully identified by nuclear magnetic resonance.

HPLC-DAD-MS/MS in negative ion mode has proved to be a powerful tool in determining flavonoid substitution patterns and thus determinant in flavonoid identification, confirming the presence of okanin and butein chalcone-aurone derivatives which are typical of the genus *Coreopsis*.

Simple and reproducible quantification methods were also developed in this project and have shown that the naturally inter-convertible chalcone-flavanone pair: Marein-Flavanomarein, are the major compounds present in both *C. tinctoria* aqueous extract and AcOEt fraction. Both, the compounds chosen for quantification and the methods themselves have been considered suitable for the quality control and characterization of *Coreopsis tinctoria* flowering tops extracts.

Considering the pharmacological approach (in vivo and in vitro studies):

*C. tinctoria* aqueous extract administration (25, 50, 100 and 300 mg/Kg) to normal (euglycemic) rats showed no significant acute antihyyperglycemic activity when compared to control, which appeared to be incoherent with this plant’s traditional use.

Further research into possible antidiabetic action of *C. tinctoria* required the extracts to be tested in a diabetic model, thus a stable low-dose glucose-intolerant rat model was developed for further subchronical adrnistration studies.

These subchronical studies revealed that both aqueous extract (500 mg/Kg; 20 mg/Kg of marein) and AcOEt fraction (125 mg/Kg; 20 mg/Kg of marein) promoted
glucose tolerance regain in the STZ glucose-intolerant rat model in three and two weeks respectively, which means that the bioactivity is probably due to more than one of the several flavonoids present in infusion and AcOEt fraction and not to marein alone.

Neither aqueous extract nor AcOEt fraction have shown hepatotoxicity (normal values of hepatic transaminases) at the concentrations tested. On the contrary, the tested flavonoid-rich AcOEt fraction promoted reestablishment of lipase values which is indicative of pancreatic function recovery.

Regarding in vitro experiments, the mouse insulinoma cell line (MIN6) model used to evaluate C. tinctoria extracts (0.1 to 1 mg/mL) insulin secretagogue activity, both as monolayers and as 3D pseudoislets and under stimulatory or substimulatory glucose levels, failed to give positive results. Additionally no cytotoxicity was observed at the tested concentrations.

Further investigations into C. tinctoria mode of antihyperglycemic activity were developed throughout this project which included testing for the cytoprotective capacity of the extracts and pure compounds at pancreatic cell level.

Results have shown that, although C. tinctoria aqueous extract, AcOEt fraction and main compound marein have shown good antioxidant capacity, the verified cytoprotective effect of C. tinctoria’s extracts and pure compounds marein and flavanomarein, is probably not directly due to an antioxidant capacity but most likely a consequence of their capacity to interact with the apoptotic signalling pathway in MIN6 cells, inhibiting it and therefore protecting pancreatic cells against cell injury.

7.2. Objectives achieved

The present study results are in accordance with the literature review (Chapter II) in which no positive correlation between a particular chemotype and antihyperglycemic activity were observed. The main compounds present in C. tinctoria flowering tops bioactive extracts are flavonoids, mainly of chalcone and flavanone structure. This type of flavonoids has been shown to act at different stages in glucose metabolism with no apparent relation between their structure and the observed activity, at present state of the art.

In this study, both chalcone marein and its correspondent flavanone have shown cytoprotective effects in pancreatic beta-cells and although no direct relation between in vitro results and in vivo can be established, the compounds could be partially

178
responsible for the claimed antidiabetic activity, through direct beta-cell protection against cell-injury observed in diabetic subjects.

So, although our initial objective of finding a dual-acting antidiabetic new chemotype (antihyperglycemic and antioxidant) was not fully achieved, the results suggest the pair marein-flavanomarein may be new antidiabetic class of compounds, since they can target injured pancreatic cells restoring insulin secretion balance.

Considering chemical characterization, *C. tinctoria* bioactive extracts were successfully characterized through chromatographic and spectroscopic techniques and suitable and adequate methods for qualitative and quantitative analysis of extracts, were developed.

Finally, regarding the study of the antidiabetic profile of *C. tinctoria* extracts and metabolites, this study has been able to establish a positive connection between *C. tinctoria* extracts administration and antihyperglycemic activity with pancreatic function recovery, supported by several *in vivo* and *in vitro* models in a pre-clinical context which does not contradict *C. tinctoria*’s traditional use in Portugal.

*C. tinctoria*’s extracts and specifically the two compounds tested *in vitro*, marein and flavanomarein, were able to preserve pancreatic beta-cell function by protecting beta-cells from apoptotic signals. This is of some interest since this type of compounds might be exploited therapeutically for type 2 diabetes as they may prevent the progressive loss of beta-cell mass, although additional experiments regarding bioavailability, metabolism and toxicity, should be performed.