

NRP Endocrine Disruptors

Final Summary

Original project title Phytoestrogens in food, food complements and medicinal plants: content and pharmacological relevance
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Isolation and pharmacological study of phytoestrogens in edible and medicinal plants.

Phytoestrogens share structural or functional similarities with estrogens and show beneficial effects on human health. This work was essentially focused in the chemical aspects of the investigations carried on phytoestrogens.

*The first study compared three species of the genus Trifolium (*T. pratense*, *T. palleescens* and *T. alpinum*) using hyphenated techniques. The first species is already on the market as food supplement for women suffering of menopausal complaints. The contents of the three species appeared to be very close. The second part of the work involved the phytochemical investigation of *Peltophorum pterocarpum*. Eight flavonoids, among which the unique flavone able to inhibit acetylcholinesterase, have been isolated and characterized, like two new peltogynoids and two new 2-phenoxychromones. Finally, estrogenic activity was characterized with a screening of natural compounds using *in vitro* biological assays. This led to a brief discussion on the phytoestrogens's structure-activity relationships and revealed three interesting active compounds.*

Research questions

Phytoestrogens are plant-derived compounds which structurally or functionally mimic mammalian estrogens and show potential benefits for human health. Many women turn to phytoestrogens as an alternative to hormone replacement therapy (HRT) because of the undesirable side-effects of synthetic estrogenic compounds, such as increased risk of breast and endometrial cancer. Although the number of publications on this topic has risen dramatically in the past couple of decades, scientific data are still lacking, particularly at the chemical, pharmacological and clinical levels. The present work aims to contribute to the research on the chemistry of phytoestrogens.

Results

The first part of the study consisted in comparing two alpine clovers (*Trifolium pallescens* Schreb. and *Trifolium alpinum* L.) with the well-known red clover (*Trifolium pratense* L.), using hyphenated techniques. The chemistry of red clover has been extensively studied and extracts are today commercially available for women suffering from menopausal complaints. The dereplication of these three species allowed the identification of several compounds from *T.pallescens* and *T.alpinum*, and revealed different quantitative distributions of secondary metabolites.

The second part of the study involved a phytochemical investigation of *Peltophorum pterocarpum* (DC.) Backer ex K.Heyne, a tropical Leguminosae, with the aim of discovering new structurally interesting derivatives of isoflavones. These compounds are undoubtedly the most active and widely studied phytoestrogens, and are almost exclusively found in plants from the Leguminosae family. The characterization of the secondary metabolites isolated led to the identification of eight simple flavonoids, never before found in the leaves of *P.pterocarpum*. Among them was the first flavone ever described as an inhibitor of acetylcholinesterase (potential for the treatment of Alzheimer's disease) and four new compounds: two peltogynoids and two 2-phenoxychromones. Finally, estrogenic activity was characterized by screening natural compounds using *in vitro* bioassays. This raised questions about the structure–activity relationship of phytoestrogens, and allowed the identification of three compounds interesting for safe HRT and on which further investigations are currently running.

Perspectives

The development of a quantitative method for the precise determination of the phytoestrogen content of the *Trifolium* extracts could be considered. It is of capital importance to quantify such endocrine disruptors and their effects, since their presence on the market as food supplements is increasing dramatically.

The new compounds isolated from *Peltophorum pterocarpum* are included in classes of compounds which are quite rare but exhibit common structural features with flavonoids. The estrogenic properties of such compounds have not yet been investigated, and this evaluation could add an interesting perspective to this work. They are currently tested in Athenes. Concerning the screening of natural compounds, it would be interesting in the future to use complementary assays, such as the stimulation of the proliferation of MDA-MB-231 breast cancer cells, which are ER-alpha-negative. Furthermore HEK-293 human embryonic carcinoma cells could be transfected with two different expression vectors for both the receptor and the luciferase reporter gene, or reporter gene expression assays realized on other types of cancer cells, such as bone cells, or brain cells, and finally *in vivo* assays could be carried out.