FACULDADE DE CIÊNCIAS
UNIVERSIDADE DE LISBOA

IBB

INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

PBRG | Plant Biotechnology Research Group

2008

Annual Report

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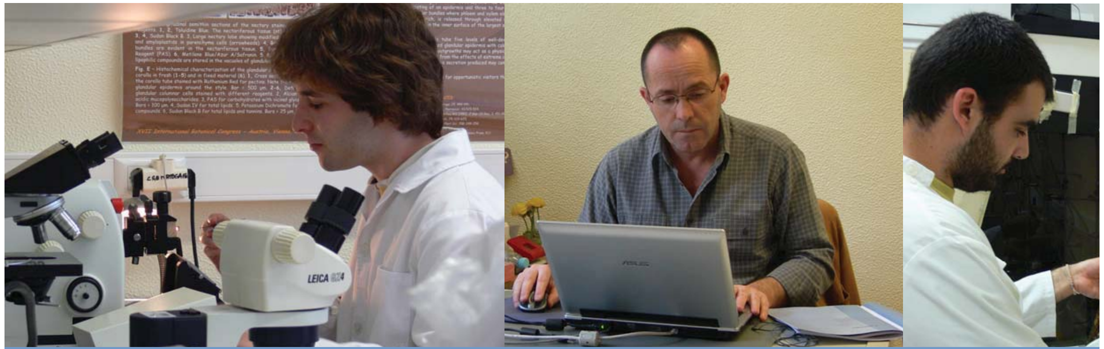

Executive Summary

The Centre for Plant Biotechnology (CPB), Faculty of Sciences, University of Lisbon, was established in 1991 and was a co-founder of the IBQF - Polo 1, in which remained integrated until the recent creation of the Institute for Biotechnology and Bioengineering (IBB), an Associated Laboratory. As part of the IBB, the CPB is the Plant Biotechnology Research Group (PBRG).

This is the first annual report of the Plant Biotechnology Research Group (PBRG) that gives an account on the major achievements obtained in 2008. As regards the Phytochemical and Molecular Analysis of endemic aromatic species, it was possible to establish a quite clear intra- and interspecific correlation between phytochemical and molecular data on *Thymus caespititius*, *Angelica lignescens* and *Melanoselinum decipiens*. Moreover, an *in vitro* screening for biological activity of the essential oils from Portuguese aromatic endemic species showed that some of them possess strong antiacetylcholinesterase activity. Relationships of the eight species of the liverwort genus *Radula* occurring in Portugal were evaluated based on molecular, phytochemical and morphological and anatomical data. Phytochemical and anatomical studies were started on pine species damaged with pine wilt disease to improve our understanding of the host-tree response mechanisms to the nematode. The assays of biotransformation of menthol and geraniol by hairy root cultures of *Anethum graveolens* and *Levisticum officinale* showed that both systems are capable of using the two substrates, although in different ways, at least for menthol. Achievements on Biology of Plant Secretion include the structure and histochemical characterization of different types of glandular trichomes from several *Plectranthus* species and the evaluation of antioxidant and antiacetylcholinesterase activities in infusions and decoctions of such species.

The recent creation of the IBB opened new possibilities for cooperation between labs with different expertise in view of the improvement of the research programmes. The young fellow researchers involved in the on going research projects held in PBRG and students from advanced education programmes will ensure the future of our expertise. Together, we will strengthen our national and international competitiveness in the area of Agro-Food Biotechnology.

José G. Barroso

PBRG Head

Plant Biotechnology Research Group | PBRG

PBRG

The Plant Biotechnology Research Group (PBRG) is a plant biology research unit integrated in the Centre for Biological and Chemical Engineering (CEBQ). CEBQ is the leading Centre of the Associated Laboratory Institute for Biotechnology and Bioengineering (IBB), a network of research centres across Portugal. IBB has been identified by the Portuguese Ministry of Science, Technology and Higher Education as a strategic infrastructure for the development of the Portuguese R&D and innovation policies in the areas of Biotechnology, Bioengineering, Biomaterials and Life, Biomedical and Agricultural Sciences. PBRG activities within the Associated Laboratory IBB are focused on the Thematic Areas of Agro-Food Biotechnology.

The involvement of PBRG in undergraduate, MSc and PhD educational programmes on advanced biotechnology and cell biology is another important and remarkable issue of its members as the senior ones are also professors with permanent positions at Faculdade de Ciências da Universidade de Lisboa. Considering the expertise of the group members, 3 research sub-programmes have been carried out and several research projects were held and have relied on the collaboration of many groups from national and international research institutions:

- **Phytochemical and Molecular Analysis (PMA)**
- ***In Vitro* Cultures (*IVC*)**
- **Biology of Plant Secretion (BPS)**

Plant Biotechnology Research Group

Objectives

Plant Biotechnology Research Group develops research in *in vitro* cultures and in particular exploits hairy roots cultures potential as natural products producing systems, namely essential oils. The research in progress, on the chemical and molecular characterization of endemic species and study of secretory structures, contributes for a better knowledge, valuation and conservation of the national plant biodiversity resources, with potential economic interest.

Research Topics

Research in PBRG relies on the expertise of the group members and three research subprogrammes have been carried out: i) Phytochemical and Molecular Analysis; ii) *In Vitro* Culture; and iii) Biology of Plant Secretion.

1. *Phytochemical and Molecular Analysis* - Evaluate the correlation between the volatiles composition and the arbitrary molecular markers and, in addition, perform the molecular characterization of terpene synthase genes aiming at clarifying how the production of terpenes, namely monoterpenes, is regulated. Identify the interesting genotypes, particularly those that produce essential oils containing bioactive compounds.

2. *In Vitro Culture* - Increase and/or modify the essential oil production by hairy root cultures by manipulation of the culture medium and of the luminosity conditions, different nitrogen sources and ratios, addition of precursors or elicitors, induction of regenerants, culturing in a bioreactor and in a two-phase system and biotransformation. Understand the hairy root culture behaviour and reveal some advantages of this *in vitro* system for the production of the different types of secondary metabolites that can be found in plant volatiles.

3. *Biology of Plant Secretion* - Survey of the different types of secretory structures, which occur in the vegetative and reproductive organs of medicinal and aromatic plants. The anatomy, differentiation and development of these structures are analyzed and the *in situ* characterization, by histochemical methods, of the main groups of compounds present in the secreted material, is performed. The ultrastructure of glandular cells is studied, for elucidation of the compartmentation of the main biosynthetic pathways of secondary metabolites and of the secretion processes.

Major Achievements

- RAPDs and volatile analyses of thirty-one individuals from *Thymus caespititius* was performed. Different clusters were obtained between molecular and volatile oil profiles and no straight correlation among collection site, chemical analysis and molecular assessments could be found.

- Chemical and genetic differences of eleven individuals of *Angelica lignescens* and three individuals of *Melanoselinum decipiens* were studied. Cluster analyses based both on the chemical composition of volatile fraction and on molecular markers grouped the fourteen accessions in two main groups, corresponding to each of the two species.

- The evaluation of biological activity of essential oils from Portuguese aromatic endemic species revealed a strong antioxidant, antimicrobial and anti-acetylcholinesterase activity, particularly for a *Thymus zygis* variety from the Northern part of Portugal.

- Hairy roots cultures of *Anethum graveolens* and of *Levisticum officinale* revealed different capacities for the biotransformation of menthol or geraniol. In both cases, substrates were rapidly metabolized.

- Trichomes of several species of *Plectranthus* were studied and their secretion histochemically characterized. From the five morphological types of glandular trichomes observed, two of them were described for the first time in Lamiaceae. The infusions and decoctions of the leaves, specially from *P. verticillatus* and *P. barbatus* showed high antioxidant and anti-acetylcholinesterase activity.

- Micromorphological studies, using scanning electron microscopy , of *Echium* species of Cape Verde Islands suggested that a taxonomic revision of this genus and of the closely related genera must be undertaken.

Selected Publications

Costa, M.M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Deans, S.G., Scheffer J.J.C., Biotechnology Letters, 30, 1265-1270 (2008).

Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Salgueiro, L., Miguel, M.G., Faleiro, M.L., Current Pharmaceutical Design, 14, 3120-3140 (2008).

Romeiras, M.M, Ascensão, L., Duarte, M.C., Dinis, M.A., Casimiro, A., Australian Syst. Bot., 21, 1-13 (2008).

Trindade, H., Costa, M.M., Lima, A.S.B., Pedro, L.G., Figueiredo, A.C., Barroso J.G., Biochem. Syst. Ecol., 36, 790-797 (2008).

PMA

Chemical polymorphism and molecular analysis of *Thymus caespititius*

Helena Trindade, A. Cristina Figueiredo, Luis G. Pedro, José G. Barroso *

The combination of chemotaxonomy and molecular biology is an approach that is being used for systematic and phylogenetic analysis of aromatic plants, replacing more traditional analysis based on morphological characters alone. Molecular biology has gained from analysis of both chloroplast and nuclear DNA sequences, which provide valuable contributions to this area of research, allowing a better understanding of plant genetic diversity and phylogenetics.

An important aromatic genus is *Thymus*, which is well known for comprising several species showing chemical polymorphism, the more important and documented one being *Th. vulgaris*. Another species with chemical polymorphism is *Thymus caespititius* (Fig.1) and at CBV plants belonging to different Portuguese populations are being characterized, using both molecular marker analysis and volatiles identification. The main objective is focused on understanding the factors responsible for the chemical polymorphism within this species, using individual accessions from Portuguese populations, in particular from Azores.

Figure 1: *Thymus caespititius*

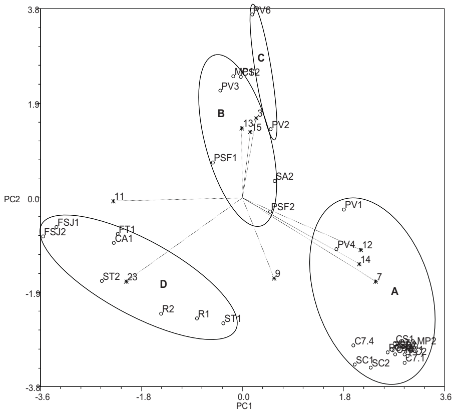

Figure 2: Principal components analysis of the volatile oil components from the 31 individuals of Th. caespititius collected on the islands of Pico, S. Jorge and Terceira (Azores). A. Thymol-rich oils, B. Carvacrol-rich oils, C. Sabinene-rich oils, D. α-Terpineol-rich oils, 3. Sabinene, 7. *p*-Cymene, 9. γ-Terpinene, 11. α-Terpineol, 12. Thymol, 13. Carvacrol, 14. Thymyl acetate, 15. Carvacryl acetate, 23. τ-Cadinol.

Chemical Polymorphism and Volatile Analysis

Different chemotypes have been identified within this species, designated according to the dominant(s) monoterpene(s). Plants which are thymol-, carvacrol-, α-terpineol- and sabinene-rich, have been identified with different possible combinations between these monoterpenes. Plants from Terceira Island were thymol-rich, while those collected on Pico yielded thymol or carvacrol-rich oils. The individuals collected on S. Jorge showed an evident chemical polymorphism, yielding volatile oils rich in thymol, carvacrol, sabinene or α-terpineol. Noteworthy is the case of five individuals collected along a 200m distance, at Pico Verde (PV) which revealed a great chemical diversity, yielding volatiles rich in sabinene, thymol or carvacrol (Fig.2). Volatile composition of the individuals confirmed that the chemical polymorphism was in some cases more evident among different plants from the same island than among those collected on different islands.

* This research program had the contribution of Monya M. Costa and A. Sofia Lima.

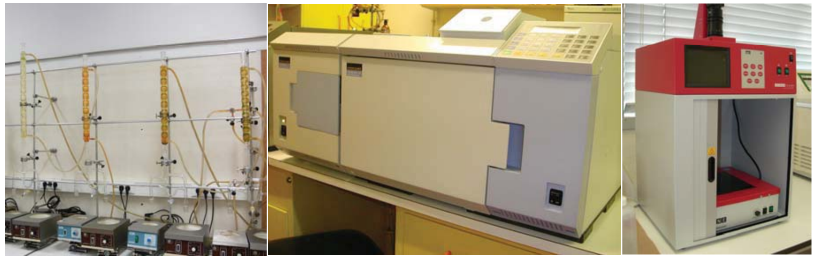

Figure 3: Hydrodistillation systems, GC-MS unit and gel image acquisition system.

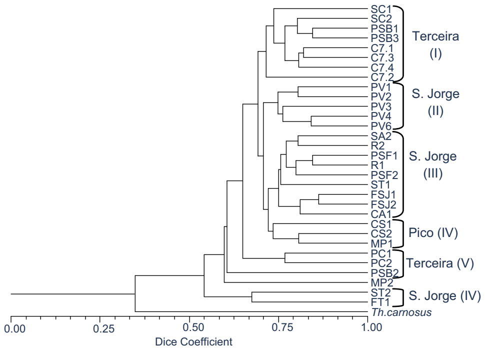

Figure 4: Dendrogram based on unweighted pair-group method with arithmetic average (UPGMA) showing the genetic similarity (Dice coefficient) among 31 *Thymus caespititius* accessions obtained by RAPD analysis. *Th. carnosus* was used as an out-group. I -VI, molecular clusters .

Molecular markers on plant analysis

Given the lack of knowledge on aromatic plants molecular biology, arbitrary markers such as RAPD (Random Amplified Polymorphic DNA) can be combined with ISSR (Inter Simple Sequence Repeat), in a combined molecular analysis. A major advantage in using these markers is that none require a previous knowledge of the genome because, in the case of RAPD, they use small arbitrary primers that amplify throughout the plant genome. In ISSR, the primers used will amplify adjacent to the ubiquitous microsatellites, taking advantage of the ubiquity of these 1-4 bases repeats in eukaryote genomes.

The selected primers were able to discriminate between individual accessions within the species, generating polymorphisms. Molecular analysis revealed in most cases that plants geographically closer were more genetically related to each other. With some exceptions, cluster analysis grouped the different accessions in accordance with the island they belonged (Fig. 4).

However, a straight correlation between volatile and molecular marker analysis could not be found, which might be due to the fact that RAPD and ISSR primers are arbitrary markers, not directly related to secondary metabolism.

Different molecular strategies have to be pursued in order to understand chemical polymorphism is this species, by using specific primers targeted towards monoterpene genes.

Hairy roots biotransformation and glycosylation capacity

A. Cristina Figueiredo, Helena Trindade, Luis G. Pedro, José G. Barroso *

Since the early 1980's hairy roots have been used as an experimental system in the production of new compounds and compounds of interest, and they are considered an alternative to cell suspension cultures for metabolites production.

Biotransformation includes a group of reactions that may contribute to increased metabolites production and to the *in vitro* production of new compounds. The biotransformation of terpenes is a process that is useful in the biological production of active terpenes and of new compounds derived there from.

As a part of an ongoing study on the secondary metabolites production capacity, *Levisticum officinale* (lovage) and *Anethum graveolens* (dill) hairy roots have been used as model systems for evaluation of biotransformation capability at the Centro de Biotecnologia Vegetal (Plant Biotechnology Center).

Hairy root cultures growth

Compared to the control cultures, the addition of the substrates (menthol and geraniol) did not markedly affect *L. officinale* and *A. graveolens* hairy roots morphology or colour. Likewise, the growth in terms of both dry and fresh weight was not influenced by the addition of substrates (Fig. 1A, C; Fig. 2A-C). Also, when growth was evaluated by the dissimilation method, no major variation was detected in the growth profile after substrates addition (Fig. 2B, D).

Constitutive volatile components

Independently of the added substrate, be it geraniol or menthol, the constitutive volatiles of lovage and dill hairy roots were always detected in variable amounts, together with the substrates and the corresponding biotransformation products, when produced. The results suggest that these hairy roots have a relatively stable production of volatile compounds, as the cultures have been maintained for over twelve years with a routine subculture every three weeks.

Biotransformation products

Following the addition of geraniol to lovage hairy roots, six new volatile compounds were detected: nerol/citronellol/neral, α-terpineol, linalool, and geranyl acetate, (Fig. 3A). The addition of geraniol to dill hairy root cultures resulted in the formation of the alcohols linalool, α-terpineol and citronellol, the aldehydes neral and geranial, the esters citronellyl, neryl and geranyl acetates and, in traces, linalool and nerol oxides, (Fig. 3B).

The capacity to transform the cyclic oxygen-containing monoterpene menthol seems to be species-specific as, unlike *L. officinale* hairy roots, *A. graveolens* hairy roots were able to convert menthol into menthyl acetate.

Glycosidic bound volatiles

After enzymatic hydrolysis with β-glycosidase, a large relative amount of menthol (45% of the total volatile oil) was detected in lovage hairy root volatiles, but still no volatile biotransformation products were detected, thus showing that all the substrate was rapidly glycosylated two weeks after addition to the culture medium. As with the menthol, both a part of the added geraniol and the biotransformation products were already found to be glycosylated 1h after addition.

In conclusion, both *L. officinale* and *A. graveolens* hairy root cultures showed biotransformation ability, although displaying a different capacity to transform the added substrates. *L. officinale* cultures also revealed a glycosylation capacity, as both the substrates and the biotransformation products were partly stored in the glycosylated form. The glycosylation capacity of lovage hairy roots can be viewed not only as a process of cellular detoxification, but also as a mechanism that transforms substrates and biotransformation products into stable and water-soluble compounds, in order to facilitate their cellular transport, storage and use in primary and/or secondary metabolism.

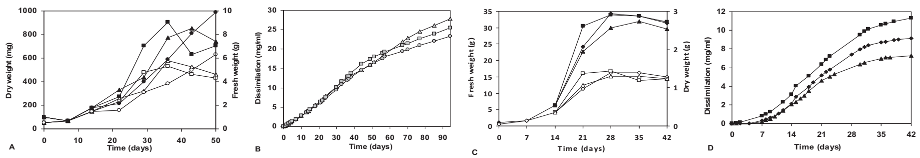

Fig. 1. (A) Dry (open symbols) and fresh weight (closed symbols) growth curves of *Levisticum officinale* hairy roots after addition of menthol or geraniol (25mg/L), and of control cultures (= without substrates). Dry weight growth curves: control (Δ), with menthol (□) and geraniol (○). Fresh weight growth curves: control (▲), with menthol (■) and geraniol (●). (B) Dissimilation growth curves of *L. officinale* hairy roots after addition of 25mg/L of menthol (■) or geraniol (●), and of control cultures (= without substrates, ▲). (C-D) Growth curves of *Anethum graveolens* hairy roots evaluated by fresh and dry weight methods (C) and by the dissimilation method (D). (C) Fresh and dry weight growth curves: control (♦, ◊, respectively], menthol (■, □) and geraniol (▲, Δ) added cultures, respectively. (D) Dissimilation growth curves: control cultures (= without substrates, ♦), menthol (■) and geraniol (▲) added cultures.

* This research program had the contribution of Inês S. Nunes (*Levisticum officinale*) and Jorge M. S. Faria (Anethum *graveolens*) as their final year graduation Master Thesis.

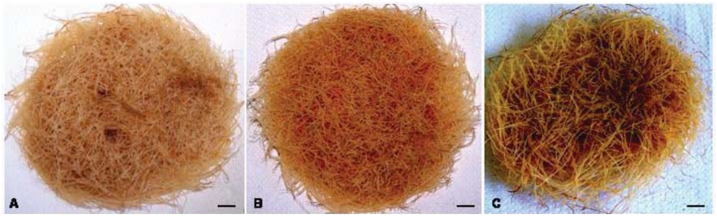

Fig. 2. General view of the control *Levisticum officinale* hairy root cultures, maintained in SH medium, in darkness, at 24 ºC and 80 r.p.m. (A), and after the addition of geraniol (B) or menthol (C) (bar = 1 cm).

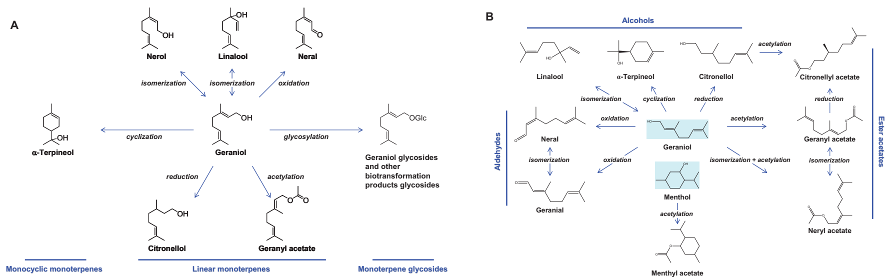

Fig. 3. A. Biotransformation products detected after geraniol addition to *Levisticum officinale* hairy roots. B. Biotransformation products detected after menthol and geraniol addition to *Anethum graveolens* hairy roots.

BPS

Secretory structures and biological activities of *Plectranthus* species*

Lia Ascensão, A. Cristina Figueiredo, Luis Pedro, José Barroso

The genus *Plectranthus* L'Herit (Lamiaceae), with about 300 species, is a large genus widespread through tropical Africa, Asia, Australia and South America. Local communities use *Plectranthus* species for many purposes, namely in the treatment of colds, coughs, sores, burns and dermatitis. Some species, as *P. barbatus* and *P. laxiflorus*, are referred by local people as therapeutically active for psychiatric problems. In order to know which cells were involved in the production of the main compounds potentially responsible for the healing properties attributed to *Plectranthus* species, the glandular structures, the essential oils and their antioxidant and antimicrobial activities were studied in *P. barbatus*, *P. ornatus* and *P. neochilus* (Fig.1). In addition, infusions and decoctions of these species and four others (*P. ecklonii*, *P. fruticosus*, *P. lanuginosus*, *P. verticillatus*) were analysed for their antiacetylcholinesterase and antioxidant activity. The main constituents of decoctions from *P. barbatus* and *P. verticillatus* were identified and their acetylcholinesterase (AChE) inhibition capacity were evaluated.

Figure 1: *Plectranthus neochilus* (smell spurflower) in the flowering period .

Glandular Structures

Five morphological types of glandular trichomes were found on the vegetative and floral organs of *P. barbatus*, *P. ornatus* and *P. neochilus*. Peltate trichomes and short capitate trichomes are similar to the previously reported to Lamiaceae, although in *Plectranthus* genus the peltate trichomes have a characteristic orange to brownish colour. Long-stalked capitate trichomes possess a two-to-three celled stalk of a variable length and a unicellular bulb-shaped head, which develops a large subcuticular space where secretion accumulates temporarily (Fig. 2A, 2C-E). Digitiform trichomes, also present on the leaves, do not show a clear distinction between the apical glandular cell and the subsidiary cells. Conoidal trichomes, which are an unusual type of glandular trichomes and are for the first time reported to Lamiaceae, have a two-to -three celled stalk and a long unicellular head (Fig. 2B). They occur exclusively on the reproductive organs, particularly on the calyx and corolla.
Histochemical characterization of the secretion products showed that peltate and long-stalked capitate trichomes (Fig. 2C-E) produce the bulk of the essential oils whereas short-stalked capitate trichomes, as well as digitiform and conoidal trichomes produce mainly polysaccharides.

Essential Oils and their Antioxidant and Antimicrobial Activities

The essential oils from the aerial organs of *P. barbatus*, *P. neochilus* and *P. ornatus,* obtained in a low yield, presented a similar composition, being monoterpene hydrocarbons the most abundant group of compounds. The main constituents, common to the three species, were α-pinene,

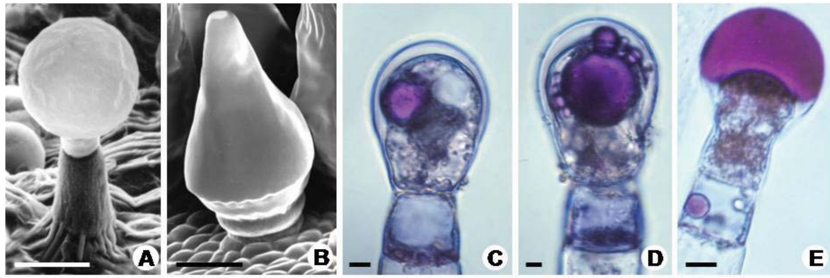

Figure 2: *Plectranthus ornatus.* **A, B -** Scanning electron micrographs showing a capitate and a conoidal trichome; **C-E -** Capitate trichomes in three developmental phases highlighting the presence of a violet-stained oleoresin (Nadi Reagent). *Bars* = 20 μm.

β-pinene, sabinene, β-caryophyllene, and 1-octen-3-ol.
The essential oils were tested for antioxidant activity using TBARS and DPPH methods and the antimicrobial activity was evaluated by the agar diffusion method. All the assayed essential oils revealed a low antioxidant and antimicrobial activity.

Acetylcholinesterase (AChE) inhibition and antioxidant activity of leaf aqueous extracts and of their main constituents

Infusions and decoctions, which are the most common forms of administration of plants in traditional therapies, were prepared from leaves of the *Plectranthus* species studied. All decoctions showed higher activities than infusions (Table1). The decoction of *P. barbatus* inhibited the activity of AChE by approximately 31.5%, when using 0.5 mg of extract/ml of test solution. This result is within the range of values found in the literature for the inhibition of this enzyme with plant extracts. *P. verticillatus* and *P. eckolonii* were the species studied that displayed the highest inhibitory activity of AChE, 59.6% and 62.8%, respectively.
The antioxidant activity of *Plectranthus* extracts, evaluated by DPPH method, was very high and similar to the antioxidant activity of BHT, a well-known synthetic standard. Both extracts of *P. verticillatus* showed the highest antioxidant activity (Table 1). Extracts of the other *Plectranthus* species also had a remarkable high antioxidant activity similar to those reported for green tea extract, a reference extract commercialised for its antioxidant activity.
These results led us to identify the main constituents of *P. barbatus* and *P. verticillatus* decoctions. Rosmarinic acid was almost the sole component of the extracts of *P. verticillatus*, whereas *P. barbatus* contained also other components (scutellarein 4'-methyl ether 7-*O*-glucuronide and (16S)-coleon E). As all of these compounds had AChE inhibition activity, their presence in the herbal teas, consumed by the populations, may explain some of the health benefits reported by the ethnobotanical studies.

Plectranthus species	AChE inhibition (%) with 0.5 mg/ml dry extract		DPPH (IC_{50} μg dry extract/ml)	
	Infusion	Decoction	Infusion	Decoction
P. barbatus	17.0 ± 5.4	31.5 ± 3.3	10.4 ± 0.3	45.8 ± 0.5
P. ecklonii	46.7 ± 0.4	62.8 ± 6.5	2.4 ± 0.1	3.8 ± 0.1
P. fructicosus	11.3 ± 1.8	31.3 ± 2.6	6.2 ± 0.9	4.4 ± 0.2
P. lanuginosus	13.7 ± 7.2	10.0 ± 1.1	5.2 ± 0.4	3.3 ± 0.2
P. verticillatus	47.4 ± 5.4	59.6 ± 7.6	1.5 ± 0.7	1.2 ± 0.4
BHT[a]	–	15.7 ± 0.2	12.0 ± 0.7	–

Table 1: Inhibition of AChE (%) and antioxidant activity of aqueous extracts of several *Plectranthus* species.

* This study is part of Luísa Mota and Pedro Falé's PhD theses supervised by the first author. The biological activities of essential oils and aqueous extracts were evaluated in collaboration with Faculdade de Engenharia e Recursos Naturais (UAlg) and Centro de Química e Bioquímica (FCUL), respectively.

Publications

Articles in International Peer-Reviewed Journals

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Rodrigues L., Monteiro, P., Póvoa, O., Teixeira, G., Moldão, M., Figueiredo, A.C., Monteiro A., "Morphology of secretory structures and essential oil composition in *Mentha cervina* L. from Portugal", Flavour Fragr. J., 23, 340-347 (2008).

Romeiras, M.M, Ascensão, L., Duarte, M.C., Dinis, M.A., Casimiro, A., "Taxonomy of *Echium* (Boraginaceae) species from Cape Verde Islands", Australian Syst. Bot., 21, 1-13 (2008).

Trindade H., Costa, M.M., Lima, A.S.B., Pedro, L.G., Figueiredo, A.C., Barroso J.G., "Genetic diversity and chemical polymorphism of *Thymus caespititius* from Pico, São Jorge and Terceira islands (Azores)", Biochem. Syst. Ecol., 36, 790-797 (2008).

Ph.D. Thesis

Ana Rita Freitas Martins Matos Fragoso, "VEGF and its receptors in B cell differentiation and in disease", PhD Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisors: Sérgio J. Dias, IPO, Lisbon, and Luis G. Pedro, FCUL, Lisbon).

Ana Paula Batista Elias, "Regulation of vascular-endothelial-growth-factor AL-TE-R$_{NATIVE}$ S^{P}-LI-C$_{ING}$ by tissue environment", PhD Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisors: Sérgio J. Dias, IPO, Lisbon, and José G. Barroso, FCUL, Lisbon).

M.Sc. Thesis

Ana Lúcia B. S. Fulgêncio "A importância da composição fenólica na cor de vinhos brancos licorosos", MSc Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisors: A. Cristina Figueiredo, FCUL, Lisbon, and Rosário Bronze, FFUL, Lisbon).

Bárbara Tavares de Moura, "Estruturas secretoras em quatro espécies da *Hypericum* (Clusiaceae) da Flora Portuguesa", MSc Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisors: Lia Ascensão, FCUL, Lisbon, and Teresa Nogueira, INETI, Lisbon).

Cláudia Cruz "Avaliação da composição química e das actividades biológicas de óleos essenciais obtidos de amostras comerciais", MSc Thesis, Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Faro (advisors: M. Graça Miguel, FERN, Faro, and A. Cristina Figueiredo, FCUL, Lisbon).

Inês Santos Nunes, "Biotransformação e regeneração em culturas de raízes transgénicas de *Levisticum officinale*: efeito no crescimento e produção de voláteis", MSc Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisor: A. Cristina Figueiredo, FCUL, Lisbon).

Jorge Miguel Silva Faria, "Produção de voláteis por culturas de raízes transgénicas de *Anethum graveolens*: influência da regeneração e da capacidade de biotransformação", MSc Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisor: Luis G. Pedro, FCUL, Lisbon).

Marta Daniela de Sá Mendes, "Caracterização química e molecular de espécies das famílias Lamiaceae e Apiaceae da flora aromática de Portugal", MSc Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisor: M. Helena Trindade, FCUL, Lisbon).

Rafaela Lacerda Santos, "Estruturas secretoras em espécies hemiparasitas de Orobanchaceae da Flora Portuguesa", MSc Thesis, Faculdade de Ciências, Universidade de Lisboa, Lisbon (advisor: Lia Ascensão, FCUL, Lisbon).

Oral Communications

International Conferences

Figueiredo, A.C., "Volatiles, hairy roots and metabolomics: what future?", Plants systems biology and medicinal plants, Society for Medicinal Plant Research (GA) Symposium, Leiden, Holanda, April (2008).

Poster Presentations

International Conferences

Dandlen, S.A., Miguel, M.G., Duarte, J.M., Faleiro, M.L., Figueiredo, A.C., Pedro, L.G., Barroso, J.G., "Effect of Portuguese *Thymus* chemical variability on antiacetylcholinesterase activity", 39th International Symposium on Essential Oils, Quedlinburg, Germany, p. 138 (2008).

Falé, P.L.V., Araújo, M.E.M., Ascensão, L., Serralheiro, M.L.M., "Acetylcholinesterase inhibition by rosmarinic acid from *Plectranthus* species", 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, Athens, Greece, PA88 in Planta Med. 74: 960 (2008).

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Other Presentations

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