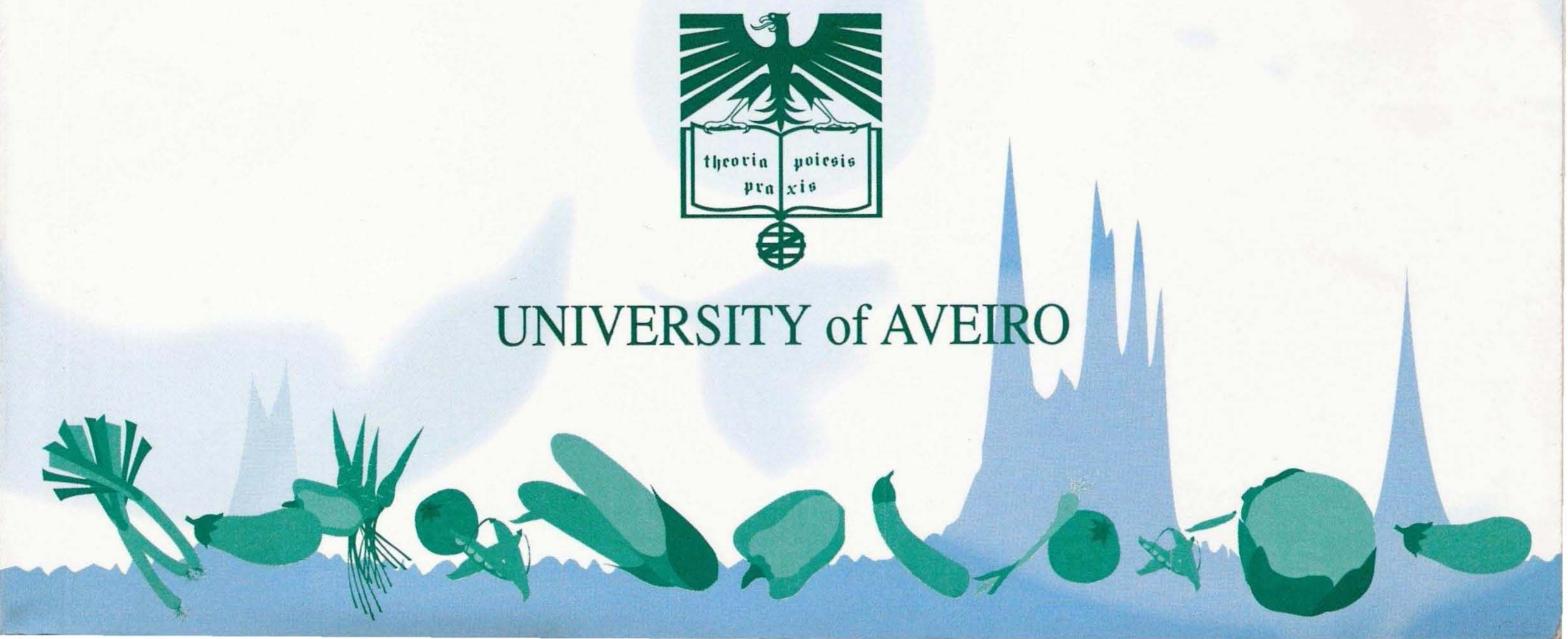
The Fifth International Conference on Applications of Magnetic Resonance in Food Science

18-20 September 2000 • Aveiro, Portugal

Book of Abstracts

UNIVERSITY of AVEIRO



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NMR STUDIES ON THE ANTIRADICALAR MECHANISM OF PHENOLIC COMPOUNDS TOWARDS 2,2-DIPHENYL-1-PICRYLHYDRAZYL RADICAL

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Phenolic compounds are the most abundant class of natural antioxidants. These types of compounds are ubiquitous in fruits and vegetables and in plant-derived beverages, such tea and wine, being important constituents of human diet. Recent studies have pointed out particular interest on phenolic type compounds (*e.g.* flavonoids, catechins, cinnamic and benzoic acid derivatives) with respect not only to the organoleptic characteristics of foodstuffs (*e.g.* flavour, bitterness, astringency) but also to their potential benefits in deleterious oxidative radicalar processes related with human disease (*e.g.* cancer, atherosclerosis).[1,2]

The antioxidant activity of phenolic compounds could be related with their antiradicalar activity and/or with the ability to act as metal ions chelators.[1,2] Although there is general agreement that catecholic compounds possess radical scavenging properties till now its mechanism of action is not fully understood.

The determination of antiradicalar potency of antioxidants is usually performed using "Trap Assays" from which a screening of their scavenging activity towards different radical species could be obtained. The DPPH method is a non-enzymatic assay widely used for this purpose, in which the reactivity of the tested compounds towards a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) is measured.[3]

Following our interest on the antioxidant behaviour of phenolic compounds[4,5] the mechanism of the antiradicalar activity of *ortho*-dihydroxy cinnamic acids and 2-styrylchromone derivatives towards DPPH$^{\bullet}$ was studied by NMR spectroscopy.

Acknowledgements: Thanks are due to FCT (I&D 62/94 and 226/94), FEDER and Praxis XXI for financial support. One of us (C.M.M. Santos) also thanks the University of Aveiro for a grant.

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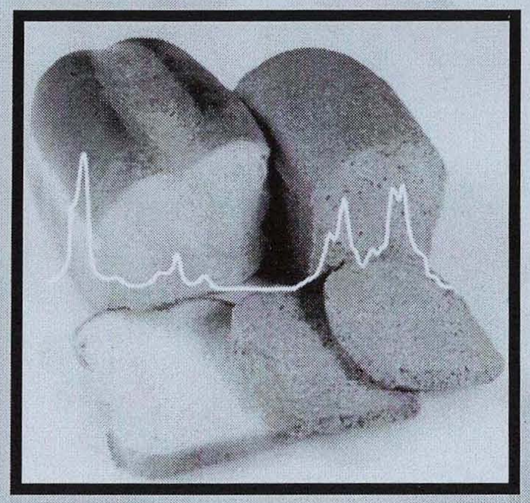

The Fifth

International Conference on Applications of Magnetic Resonance in Food Science

CERTIFICATE OF ATTENDANCE

This is to confirm that

SANTOS, CLEMENTINA

attended the **Fifth International Conference on Applications of Magnetic Resonance in Food Science** that was held in the **University of Aveiro** from 18-20 September 2000.

The Organisation

Artur Silva

NMR Studies on Antiradicalar Mechanism of Phenolic Compounds Towards 2,2-Diphenyl-1-picrylhydrazyl Radical

Artur M. S. Silva[a], Clementina M. M. Santos[a], José A. S. Cavaleiro[a],
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INTRODUCTION

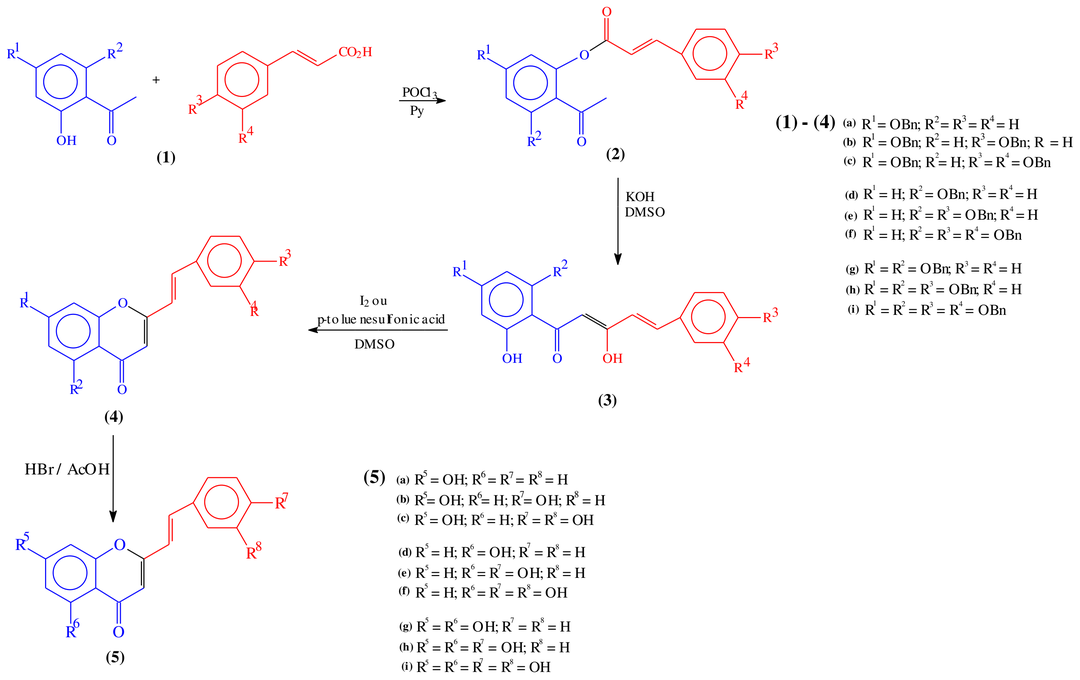
The evaluation of antioxidant vs. antiradicalar activity of phenolic compounds, either of natural or synthetic origin, is nowadays an important area of research in the field of food and medicinal sciences.

Phenolic type compounds (e.g. flavonoids, catechins, cinnamic and benzoic acid derivatives) have been extensively investigated not only to the organoleptic characteristics of foodstuffs (e.g. flavour, biterness, astringency) but also to their benefits in oxidative radicalar processes related with human desease (e.g. cancer, atherosclerosis and aging) [1, 2].

The evaluation of antiradicalar potency of antioxidants is usually performed using "trap assays" from which a screening of their scavenging activity towards different radical species could be obtained. The DPPH method is a non-enzymatic assay widely used with this purpose [3].

Following our interest on the antioxidant behaviour of phenolic compounds [4, 5] the mechanism of the antiradicalar activity of ortho-dihydroxy cinnamic acids and 2-styrylchromones derivatives towards DPPH• was studied by NMR spectroscopy.

SYNTHESIS OF HYDROXY-2-STYRYLCHROMONES



(1) - (4)
(a) R^1= OBn; R^2= R^3= R^4= H
(b) R^1 = OBn; R^2= H; R^3= OBn; R = H
(c) R^1 = OBn; R^2= H; R^3= R^4= OBn
(d) R^1 = H; R^2 = OBn; R^3 = R^4 = H
(e) R^1 = H; R^2 = R^3 = OBn; R^4 = H
(f) R^1 = H; R^2 = R^3 = R^4 = OBn
(g) R^1 = R^2 = OBn; R^3 = R^4 = H
(h) R^1 = R^2 = R^3 = OBn; R^4 = H
(i) R^1 = R^2 = R^3 = R^4 = OBn

(5)
(a) R^5 = OH; R^6 = R^7 = R^8 = H
(b) R^5= OH; R^6= H; R^7= OH; R^8 = H
(c) R^5 = OH; R^6 = H; R^7 = R^8 = OH
(d) R^5 = H; R^6 = OH; R^7 = R^8 = H
(e) R^5 = H; R^6 = R^7 = OH; R^8 = H
(f) R^5 = H; R^6 = R^7 = R^8 = OH
(g) R^5 = R^6 = OH; R^7 = R^8 = H
(h) R^5 = R^6 = R^7 = OH; R^8 = H
(i) R^5 = R^6 = R^7 = R^8 = OH

EVALUATION OF THE ANTIRADICALAR ACTIVITY

General procedure

The free radical scavenging activity of the tested compounds were measured using DPPH radical method. The experimental procedure was adapted from Ohnishi et al..[2] The reaction mixture containing a total volume of 2,5 ml: 2 ml of 0,1 mM DPPH• (in 10% DMSO and 90% EtOH) and 0,5 ml of the test compound (in 10% DMSO and 90% EtOH). The reduction of DPPH• was followed by monotoring the decrease of absorbance at 517 nm for 20 minutes. The scavenging activity was measured as the decrease of the absorvance of the DPPH• expressed as a % of the absorbance of a control solution without test substances. The mean value was obtained from triplicate experiments.

REFERENCES

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[2] Flavonoids in Health and Disease, Eds. C. A. Rice-Evans and L. Packer, Marcel Dekker, New York, **1998**.
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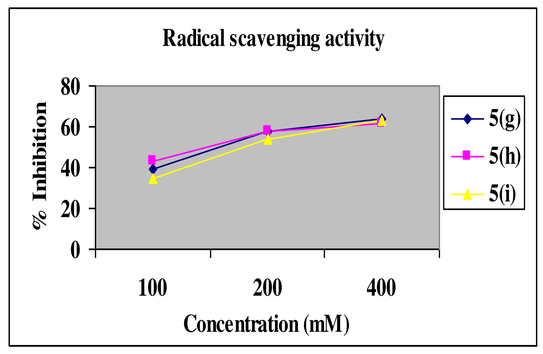
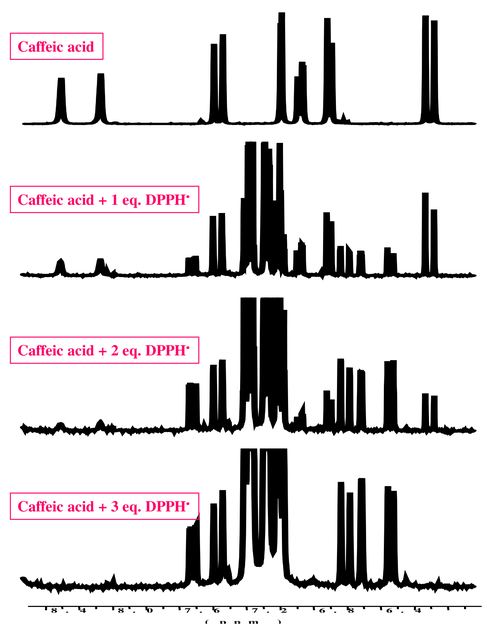
Results and discussion

Inhibition percentage of DPPH• (SD)

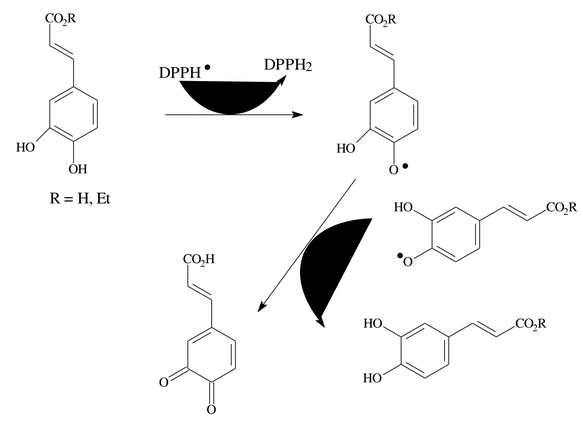
Cinnamic acid	4-hydroxycinnamic ac.	3,4-dihydroxyicinnamic ac.
0,7±0,2	0,9±0,3	10,5±0,4
Compound **(5a)**	Compound **(5d)**	Compound **(5g)**
0,4±0,2	4,4±0,4	19,3±1,5
Compound **(5b)**	Compound **(5e)**	Compound **(5h)**
0,4±0,1	2,2±0,5	31,1±1,6
Compound **(5c)**	Compound **(5f)**	Compound **(5i)**
0,4±0,2	3,3±0,6	23,2±1,3

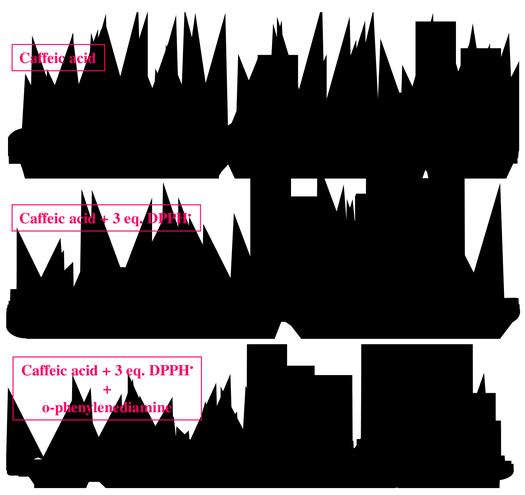




^{1}H NMR spectra of caffeic acid and their reaction mixtures

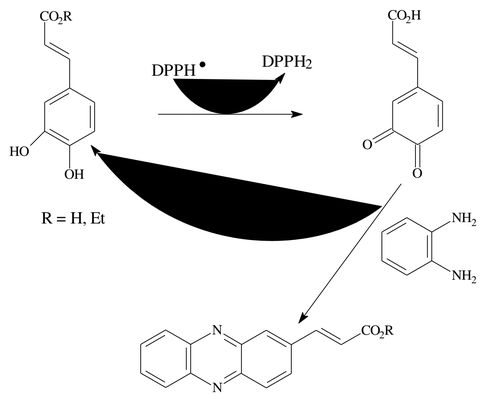
Mechanism of the antiradicalar activity of ortho-dihydroxy compounds





^{13}C NMR spectra of caffeic acid and their reaction mixtures

"Trapping" the *ortho*-benzoquinone with phenylenediamine



Small amounts

The mechanism of the antiradicalar activity of ortho-dihydroxy compounds was proved by NMR.

The behaviour of 2-styrylchromones **[5(g)-(i)]** was similar to those of caffeic acid dericatives and the NMR results were also similar.

Almost no decrease in absorbance occurred with the addition of **5(a)** or **5(b)** while the compounds **5(c)-(f)** showed only a slight decrease. However, the presence of the 3',4'-dihydroxy substituents on the B ring of 2-styrylchromones **[5(g)-(i)]** demonstrated the highest inhibition efficiency.

DPPH radical scavenging activities of these compounds increased dose-dependently of the concentration. The more active compounds **[5(g)-(i)]** were tested at concentrations 100, 200 and 400 mM and showed an increase in scavenging activity.

ACKNOWLEDGEMENTS

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