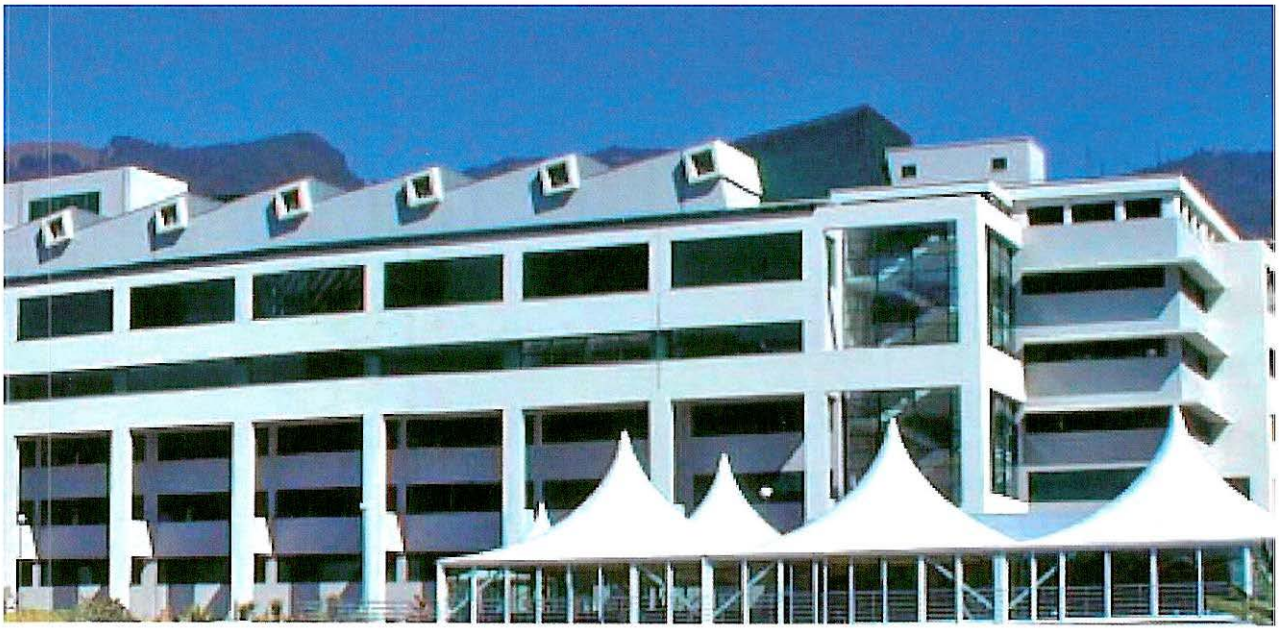

6º Encontro Nacional de Cromatografia

Universidade da Madeira
2009

UNIVERSIDADE da MADEIRA

CQM CENTRO DE QUÍMICA DA MADEIRA

P.34 CHROMATOGRAPHIC ANALYSIS OF IMPORTANT PHYTOCHEMICALS IN AROMATIC PLANTS

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The number of individual phytochemicals already identified in fruits and vegetables is estimated in >5,000, but a large percentage still remain unknown and need to be identified before we can fully understand the health benefits of phytochemicals in whole foods[1]. However, more and more convincing evidence suggests that the benefits of phytochemicals may be even greater than is currently understood, because the oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases[2]. Phytochemicals can include secondary metabolites such as phenolic compounds but also vitamins, sugars and fatty acids, and these compounds can be used as nutraceuticals.

Cistus ladanifer and other species of *Cistaceae* are used as general remedies in folk medicine for treatment of various skin diseases, as antidiarrheics, and as anti-inflammatory agents[3]. According to literature the foliage of *Cupressus lusitanica* is used in indigenous practices to treat catarrh, headache and dermatitis. The essential oil of the leaves is used against rheumatism, whooping cough, and styptic problems[4]. For the production of phytopharmaceuticals, essential oils rich in 1,8-cineole (called also "eucalyptol"), are of special importance. These products are applied for relief of head colds, rheumatism, muscular pain, and as expectorant in cases of bronchitis (added to cough syrups).

In the present work, the phytochemical composition of three aromatic plants (*Cistus ladanifer L.*, *Cupressus lusitanica Mill.* and *Eucalyptus gunnii Hook.* f) were evaluated, in order to valorize them as sources of nutraceuticals. Samples were analyzed for ascorbic acid by spectrophotometric assay, tocopherols by high performance liquid chromatography (HPLC) coupled to a fluorescence detector, sugars by HPLC coupled to a refraction index detector (RID), and fatty acids by gas-chromatography (GC) coupled to a flame ionization detector (FID).

For tocopherols analysis it was used a simple solid-liquid extraction procedure without saponification step and the chromatographic separation was achieved with a a YMC-Pack Polyamine II column (250x4.6mm) operating at 30°C (7971R Grace oven), using a Knauer Smartline HPLC equipment with a 2500 UV detector at 295nm (Knauer, Germany) connected in series with a FP-2020 fluorescence detector (Jasco, Japan) programmed for excitation at 290nm and emission at 330nm.5 The mobile phase used was hexane/ethyl acetate (70:30, v/v) at a flow rate of 1.0mL/min, and the injection volume was 20μl. For sugars analysis it was used a solid-liquid extraction procedure and the chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6mm x 250mm, 5mm, Knauer) operating at 35°C, using a Knauer Smartline HPLC equipment with RID[5]. The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1mL/min, and the injection volume was 20μl. The fatty acid profile was analyzed, after a trans-esterification procedure, with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID and a Macherey-Nagel column (30m x 0.32mm ID x 0.25μm df)[5]. The oven temperature program was as follows: the initial temperature of the column was 50°C, held for 2min, then a 10°C/min ramp to 240°C and held for 11min. The carrier gas (hydrogen) flow-rate was 4.0mL/min (0.61 bar), measured at 50°C. Split injection (1:40) was carried out at 250°C.

Eucalyptus sample presented the highest content of tocopherols (1558.27μg/g of dry weight). Otherwise, ascorbic acid was the most abundant vitamin in *Cupressus lusitanica* and *Cistus ladanifer* leaves, and particularly for the latter sample it presented a very high level (647.64mg/g). The aromatic plants presented fructose, glucose, sucrose and raffinose as main

sugars. *Cupressus lusitanica* revealed the highest total sugars content (82.96mg/g), and highest levels of fructose and raffinose. The present study describes for the first time the sugars composition in these aromatic plants. The major fatty acids found in *Cupressus lusitanica* and *Eucalyptus gunnii* samples were α-linolenic acid (C18:3), followed by linoleic acid (C18:2) and palmitic acid (C16:0). For *Cistus ladanifer* leaves, eicosadienoic acid (C20:2) was the most abundant fatty acid, and was followed by arachidic acid (C20:0) and α-linolenic acid. Twenty four fatty acids were identified and quantified. As far as we know, nothing has been reported on fatty acid composition of the leaves of the three aromatic plants, despite the extensive reports on their essential oils composition[4,6].

Overall, the combination of the useful phytochemicals found in the analysed aromatic plants with the precious contribution of vitamins (tocopherols and ascorbic acid) and reducing sugars (glucose and fructose) make them a possible source of compounds to be used as remedies for diseases related to oxidative stress, or dermatological applications, as also for cosmetics. The polyunsaturated fatty acids (linoleic, α-linoleic and eicosadienoic acids) including omega-3 and omega-6 families detected in the plants constitute another important class of phytochemicals due to their generalised beneficial health effects.

Acknowledgements

Foundation for Science and Technology (Portugal) for financial support to L. Barros (SFRH/BPD/4609/2008).

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