Composition of lipophilic extracts from barks of different eucalypt species

Joana Ferreira: Ph, ISA, Portugal, jpferreira@isa.ulisboa.pt
Jorge Gominho: PhD, ISA, Portugal, jgominho@isa.ulisboa.pt
Isabel Miranda: PhD, ISA, Portugal, imiranda@isa.ulisboa.pt
Helena Pereira: Professor, ISA, Portugal, hpereira@isa.ulisboa.pt

Abstract

The barks of different species show a large diversity in chemical composition, but are generally characterized by having a substantial content of non-structural organic components that can be solubilized by appropriate solvents. These extractives vary between species in total content as well as in their composition. Today these components are being viewed with a renewed interest as they can be a source of potential interesting chemicals and bioactive drugs.

In this work we study the barks of 12 eucalypt species: *Eucalyptus botryoides*, *E. camaldulensis*, *E. globulus*, *E. grandis*, *E. maculata*, *E. ovata*, *E. propinqua*, *E. resinifera*, *E. rudis*, *E. saligna*, *E. sideroxylon*, and *E. viminalis* which have a substantial content in extractives. The lipophilic extracts that were solubilized by dichloromethane extraction were studied here. They consisted of the smallest fraction of extractives (i.e. ethanol and water solubles represented a higher proportion of the total extractives) and ranged between these eucalypt species as 1-2% of the oven-dry mass.

The lipophilic extractives of the studied barks are mainly composed by triterpenes (55%) namely betulinic acid, ursolic acid, maslinic acid, corosolic acid and oleanolic acid. Fatty acids exist as minor compounds (12.3% on average), being C16 and C18 the most common. β-Sitosterol and stigmastanol are the most abundant sterols found in the bark extracts, in an overall content of 6.8% of total identified compounds.

All the bark extracts went under alkaline hydrolysis in order to identify possible esterified structures; the higher content in fatty acids found in most bark extracts after hydrolysis confirmed the existence of polymerized structures in the lipophilic extracts.

Keywords: eucalyptus; lipophilic extractives; triterpenes; GC/MS analysis.

Introduction

Eucalyptus species have nowadays an important role in the fulfillment of the worldwide increasing demand for pulpwood.[1] Eucalyptus species are the most important fiber sources for pulp and paper production in South-West Europe (Portugal and Spain), South America (Brazil and Chile), South Africa, Japan and other countries.[2] The increasing interest in several Eucalyptus species as wood sources for pulp production is mainly related to their rapid growth and to their behavior during pulping and bleaching, as well as to the excellent properties of the final pulps.[2-6] Some of these residues and by-products can be sources of valuable compounds – such as phytosterols,[7] lignans [8-10] and triterpenoids.[11,12] The integrated exploitation of some of these compounds in pulp mills is viewed as one of the most successful examples of the biorefinery concept implementation in this industrial branch.[13]

Some previous studies were devoted to the lipophilic composition of bark in some of the most important Eucalyptus species used by pulp industry worldwide, namely *E. globulus*, [3,11,1] *E. grandis*, *E. urograndis* and *E. maidenii*,, as well as other biomass residues obtained from *E. globulus*.[15,16] We know from the literature that bark is among the most interesting residues for possible exploitation in an integrated approach.[17] It is also reported that the lipophilic extracts from the outer barks of all these Eucalyptus species, mostly *E. globulus*, contain high amounts of triterpenic acids,[3,15,16] that show promising nutraceutical and pharmacological properties, due to their antitumoral [17,18] and anti-angiogenic [18] properties, or as precursors for anti-HIV drugs, some of them already in clinical trial phase.[19] Considering the future perspectives for these triterpenic molecules, the search for biomass sources in which they can be abundantly found becomes an important issue.

The work summarizes the findings of our research group focusing the lipophilic extract composition of 12 eucalypt species.

Experimental

Samples

The bark samples for 4-year-old trees from *Eucalyptus botryoides*, *E. camaldulensis*, *E. globulus*, *E. grandis*, *E. maculata*, *E. ovata*, *E. propinqua*, *E. resinifera*, *E. rudis*, *E. saligna*, *E. sideroxylon*, and *E. viminalis* trees were collected from an eucalypt arboretum located in the fields of the School of Agriculture, University of Lisbon (ULisboa), at Tapada da Ajuda, Lisboa, Portugal (38°42′N; 09°10′W). Bark can be easily separated in two distinct morphological regions: the outer and the inner bark;[19] the inner and outer fractions were hand separated from fresh bark and air dried; representative samples of outer bark fractions were then collected, ground and sieved, and the granulometric fractions of 40–60 mesh were used for analysis.

The milled samples were Soxhlet extracted with dichloromethane for 6 h. The solvent was evaporated to dryness and the extracts were weighed.

Alkaline hydrolysis

For the alkaline hydrolysis, 2 mg of each extract were dissolved in 10 ml of 1 M KOH in 10% aqueous methanol. The mixture was heated at 100 °C, under nitrogen atmosphere, for 1 h. The reaction mixture was cooled, acidified with 1 M HCl to pH~2 and then extracted three times with dichloromethane. The solvent was evaporated to dryness.

GC-MS analyses

Before GC-MS analysis, nearly 2 mg of each dried sample were trimethylsilylated according to the literature (Ekman 1983a). Each sample was injected three times. GC-MS analyses were performed using a GC–MS (Agilent 5973 MSD) with the following GC conditions: Zebron 7HG-G015-02 column (30 m, 0.25 mm; ID, 0.1 µm film thickness), injector 400ºC, oven temperature program, 50ºC (1 min), rate of 10ºC/min up to 150ºC, rate of 4ºC/min up to 300ºC, rate of 5ºC/min up to 370ºC, rate of 8ºC/min up to 380ºC (5 min). The MS source was kept at 220ºC and the electron impact mass spectra (EIMS) taken at 70 eV of energy.

Compounds were identified as TMS derivatives by comparing their mass spectra with a GC–MS spectral library (Wiley, NIST), and by comparing their fragmentation profiles with published data. [21] For semi-quantitative analysis, the area of peaks in the total ion chromatograms of the GC–MS analysis was integrated and their relative proportions expressed as percentage

Results and Discussion

The dichloromethane extractives composition from barks of the 12 eucalypt species have three main chemical families of compounds: triterpenes, sterols and fatty acids (Fig.1).

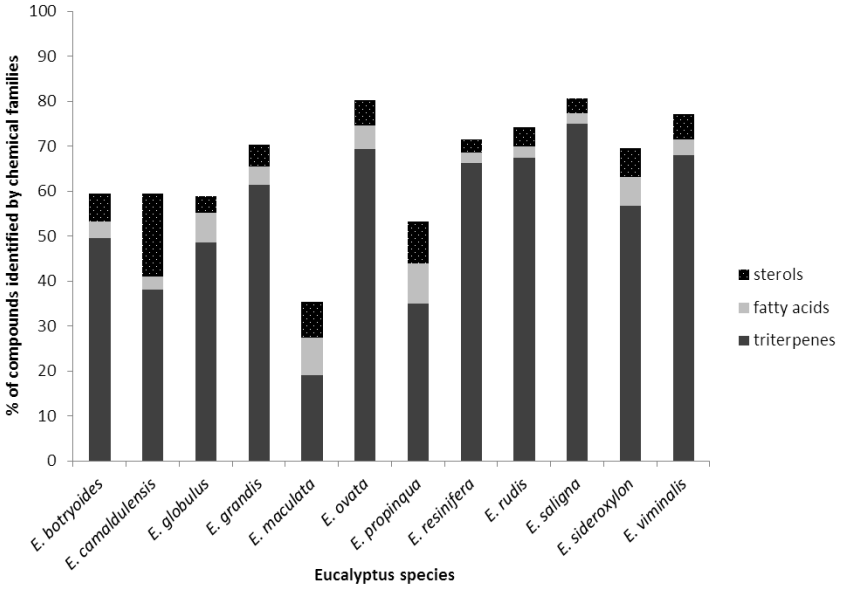

Figure 1 – Composition of the lipophilic extracts from the bark from 12 eucalypt species regarding the main chemical families of the compounds found in GC/MS analysis.

Triterpenes constitute the major lipophilic compounds identified in all the studied eucalypt species (around 55% of the identified compounds). Triterpenic acids as betulinic acid, maslinic acid, oleanolic acid, corosolic acid and ursolic acid were the most abundant compounds. In *E*. *maculata* bark there are still some unidentified compounds, so the percentage of identified compounds is less (around 50%) than for the other species. Triterpenic content is higher in the bark of *E. saligna*, followed by *E. ovata*, *E viminalis* and *E. rudis* (67-75%).

Fatty acids and sterols exist as minor compounds (12.3 and 6.8%, respectively). The most commonly identified fatty acids are C16 and C18. β-Sitosterol and stigmastanol are the most abundant sterols, with lower contents comparing to the ones found in wood.[3,5,19,20] Stigamats-4-en-3-one, stigmastan-3,5-diene and campesterol were also identified. The highest content in sterols was found for *E. camaldulensis* bark.

Upon alkaline hydrolysis there was an increase in the amounts of aliphatic extractives and particularly in the amount of fatty acids. This is indicative that a fraction of these compounds is present in the dichloromethane extracts in esterified forms making up polymerized structures e.g. dimers that could not be detected by the GC analysis in the solution. This fact can also be assigned to the hydrolysis of suberin (of periderm) and cutin (of epidermis) or their related waxes, since the identified fatty acids and alcohols are common components of such structures.[22,23]

With the exception of *E. ovata* and *E. rudis* barks, the total amount of fatty acids and triterpenes identified in the lipophilic extracts is higher after alkaline hydrolysis (AH) that before hydrolysis (BH), (Fig. 2), again probably because in the majority of the barks studied, triterpenes are esterified with long-chain fatty acid molecules, organized in polymeric structures not detected by GC/MS of the solution.

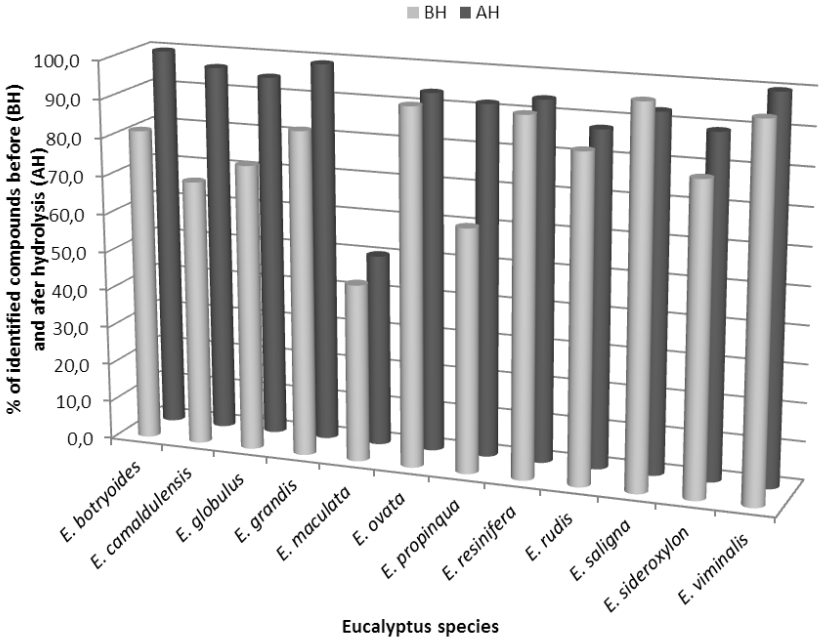

Figure 2 – Composition of the lipophilic extracts by chemical families identified in GC/MS before (BH) and after hydrolysis (AH).

Conclusions

The composition of lipophilic extractives from the bark of 12 eucalypt species was studied by GC/MS analysis. It is generally concluded that eucalyptus barks are high quality sources of lipophilic chemicals, namely triterpenes and fatty acids.

Specific conclusions include:

- The chemical composition of the outer bark extracts from the studied species is quite similar as regards chemical families' content.
- The extracts are mainly composed of triterpenes.
- As to the hypothetical exploitation of bark for triterpenes production all the eucalypt species seem to be promising raw materials.

References

1. Forrester, D. I., Theiveyanathan, S., Collopy, J. J., Marcaret, N. E., Forest Ecol. Manag. 259 (9): 1761 (2010).
2. Rencoret, J., Gutierrez, A., del Rio, J. C., Holzforschung 61 (2): 165 (2007).
3. Freire, C. S. R., Silvestre, A. J. D., Pereira, C. C. L., Pascoal Neto, C., Cavaleiro, J. A. S., J. Wood Chem. Technol. 22 (1): 55 (2002).
4. Freire, C. S. R., Pinto, P. C. R., Santiago, A. S., Silvestre, A. J. D., Dmitry, V. E., Pascoal Neto, C., Bioresources 1 (1): 3 (2006).
5. Gutierrez, A., del Rio, J. C., González-Vila, F. J., Martín, F., Holzforschung 53 (5): 481 (1999).
6. Pereira, H., Miranda, I., Tavares, F., Gominho, J., Quilhó, T., Graça, J., Rodrigues, J., Shatalov, A., Knapic, S., Qualidade e utilização tecnológica do eucalipto (Eucalyptus globulus). Centro de Estudos Florestais, Lisboa, Portugal (2009).
7. Fernandes, P. and Cabral, J. M. S., Bioresource Technol. 98 (12): 2335 (2007).
8. Pietarinen, S. P., Willför, S. M., Ahotupa, M. O., Hemming, J., Holmbom, B. R., J. Wood Sci., 52 (5): 436 (2006).
9. Willför, S. M., Nisula, L., Hemming, J., Reunanen, M., Holmbom, B., Holzforschung, 58 (4): 335 (2004).
10. Willför, S. M., Nisula, L., Hemming, J., Reunanen, M., Holmbom, B., Holzforschung, 58 (6): 650 (2004).
11. Kolomitsyn, I. V., Holy, J., Perkins, E., Krasutsky, P. A., Nat. Prod. Commun. 2 (1): 17 (2007).
12. Krasutsky, P. A., Nat. Prod. Rev. 23: 919 (2006).
13. Huang, H.-J., Ramaswamy, S., Tschirner, U. W., Ramarao, B. V., Sep. Purif. Technol. 62 (1): 1 (2008).
14. Miranda, I., Gominho, J., Pereira, J., Bioresources 7 (3): 4350 (2012).
15. Domingues, R. M. A., Sousa, G. D. A., Freire, C. S. R., Silvestre, A. J. D., Pascoal Neto, C., Ind. Crops Prod. 31 (1): 65 (2010).
16. Domingues, R. M. A., Sousa, G. D. A., Silva, C. M., Freire, C. S. R., Silvestre, A. J. D., Pascoal Neto, C., Ind. Crops Prod. 33 (1): 158 (2011).
17. Laszczyk, M. N., Planta Med. 75 (15): 1549 (2009)
18. Liu, J., J. Ethnopharmacol. 100 (1-2): 92 (2005).
19. Sogno, I., et al., Procs. 5th International Cancer Prevention Conference, St. Gallen, Switzerland, (2009).
20. Smith, M. G., Gianoulis, T. A., Pukatzki, S., Mekallanos, J. J., Ornston, L. N., Gerstein, M., Snyder, M., Genes Dev., 21 (5): 601 (2007).
21. Kolattukudy, P. E. and Espelie, K. E., Chemistry, biochemistry and function of suberin and associated waxes. In: Natural Products of Woody Plants (Ed: J. W. Rowe), Springer, Berlin, Heidelberg, New York, (1989).
22. Swan, B. and Âkablom, I.-S., Svensk. Papperstidn. 70: 239 (1967).
23. Wallis, A. F. A., and Wearne, R. H., Procs. 51st Annual General Conference Appita, Melbourne, Australia (1997).

Acknowledgements

This work was supported by the Strategic Project (UID/AGR/00239/2013) of Centro de Estudos Florestais, a research unit supported by the national funding of FCT – Fundação para a Ciência e a Tecnologia.