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LIPOPHILIC EXTRACTS FROM EUCALYPT AND ACACIAS BIOMASS – A VALORIZATION ROUTE UNDER THE BIOREFINING CONTEXT

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SUMMARY

The lipophilic extractives soluble in dichloromethane of *Eucalyptus globulus* (wood, stumps wood and bark) and *Acacia melanoxylon* (wood and crown residues) were studied by GC-MS to access the potential to further valorize the first species and to get a return to mitigate control costs for the latter species. Lipophilic extracts represented 0.3-0.6% of the chemical composition of *E. globulus* biomass. Wood was rich in fatty acids and phytosterols, while the main families of stumps wood and bark were triterpenoids and fatty acids. β-Sitosterol was the main phytosterol identified (542mg/kg stump wood) and asiatic and arjunolic acid the main triterpenoids (220 and 183 mg/kg of stump wood respectively) with interesting pharmacological properties. Regarding the *A. melanoxylon* biomass residues, extracts soluble in dichloromethane represented 0.6 and 1.8% of the chemical composition for wood and crown residues respectively. Spinasterol with anti-inflammatory and anti-tumour properties, was the main compound of both biomasses with 542.2mg/kg of wood and 1157.6mg/kg of crown residues.

Keywords: Bioeconomy, Extractives, Forest residues, GC-MS analysis, Zero-waste philosophy.

INTRODUCTION

Eucalypt and acacias species are available in Portugal in large amount, the first species because it is the raw material for the pulp&paper industry and the second because it has been considered an invasive species due to its dominance over the natural flora and rapid proliferation, in particular after forest fires, leading to great losses of flora and fauna biodiversity. The management of acacias involves several steps, such as mechanical removal (debarking or cutting the tree) followed by a chemical step using a pesticide or biological control to prevent the coppice sprouting. However, all these operations are very expensive, and stakeholders don´t get any return from them, meaning no incentives to carry on these operations in the long-term, and consequently aggravating the spreading of acacias over the years. One way to overcome this problem would be the valorization of acacia biomass for value-added products in a cascade view with the only objective of minimizing the control operation costs of stakeholders. For the *Eucalyptus globulus* biomass, the same could be applied prior to the pulping process, to further valorize this biomass.

The first valorization process that can be applied in lignocellulosic biomass without the destruction of the plant cell wall is the extraction with different solvents to obtain sources of compounds that can be used for various purposes. *Acacia melanoxylon* (main leaves or mixture of aerial parts) has been studied, in particular the polar extracts, revealing their phytochemical and antioxidant or anti-inflammatory properties [1,2] and the non-polar extracts have been studied by [3,4]. However, there is little information on the lipophilic extracts in *acacia* wood. In this work the non-polar extracts soluble in dichloromethane of *Eucalyptus* and *Acacia melanoxylon* biomass were studied by GC-MS to identify the compounds present to determine the potential of these extracts to be valorized.

EXPERIMENTAL

Eucalyptus globulus trees and stumps were donated by Altri; and *Acacia melanoxylon* residues were donated by Parques de Sintra-Monte da Lua (chips, crown residues). All the samples were aid dried until constant moisture content, milled and Soxhlet-extracted using dichloromethane (DCM) for 8h. Then the solvent was evaporated in a rotavapor at 40ºC, and the lipophilic extracts transferred to a pre-weighted 5 mL vial and dried (vacuum oven) for yield determination. The results were expressed as oven dry material.

For the *Eucalyptus globulus* samples extracts around 1 mg of DCM extract was derivatized to trimethylsilyl ethers/esters (TMS) with 120 µL of pyridine and 80 µL of BSTFA and oven heated for 30 min at 60ºC. The extracts were analysed by GC-MS (Agilent GC 7890A coupled to 5975C MSD) under the conditions described elsewhere [5].

The *Acacia* extracts were analysed in a GC-MS (Thermo Trace Ultra Polaris Ion Trap apparatus) with a manual split/splitless injector and a Phenomenex capillary column ZB-35HT (30mx0.25mmx0.10µm). Chromatogram peak areas were taken by automatic integration (Thermo Excalibur software), with manual corrections when necessary. The derivatezed samples were analyzed following the parameters: injection of 2 µl in split mode (1:20), temperature of 280ºC for the injector and interface, 0.8 ml/min of helium carrier gas and a GC-oven program temperature of 50ºC (held 2 min), 10ºC/min to 200ºC, 5ºC/min to 370ºC (held 5 min). For mass spectra analysis, electron impact ionization at 70 eV, 230ºC for the ion source temperature and 0.3 ml/min of damping helium gas were used. The identification of compounds was made by comparing the mass spectral with Wiley 6, NIST and private spectra collection libraries.

RESULTS AND DISCUSSION

Table 1 presents the extractives content in both species' biomass. In eucalypt, total extractives varied according to the biomass: in stem wood ranged from 6.2% to 12.5%, with a mean value of 9.4% [6]; while in stumps wood the range was between 11.3 to 17.4% depending on the collected site, with mean value of 15.1% [7]; and stumps bark had 4.1-7.5% [5]. The lipophilic extracts attained by dichloromethane extraction (DCM) were not so different in the case of eucalypt biomass ranging from 0.3 to 0.6% which is the usual range of values found in literature for this species [8]. *Acacia* residues were constituted by wood chips with a total extractives of 7.3% with 0.6% soluble in DCM and crown biomass, which attained the higher content of total extractives (13.3%) as well as DCM extracts (1.8%). Other study mentioned an average of total extractives in acacia sapwood 4.1% and in heartwood 8.1%, while the DCM extracts represented 0.4% in both tissues [9]. The DCM extracts of branches and leaves represented only 1.1% (17.63g in 1250g of biomass) as reported by Alves et al. [2], which is not far from the result of crown samples reported here.

Table 1. Total extractives and lipophilic contents as % of oven dry material.

Samples		Total extractives (% of o.d. material)	Lipophilic fraction (DCM)*	reference
Eucalyptus globulus	Stem wood	6.2-12.5	0.3-0.5	[6]
	Stumps wood	11.3-17.4	0.4	[7]
	Stumps bark	4.1-7.5	0.6	[5]
Acacia melanoxylon	chips	7.3	0.6	-
	crown	13.3	1.8	-

(*) – dichloromethane extracts.

Figure 1 shows an example of DCM extract chromatogram of eucalypt wood. It can be seen that the chromatogram presents many peaks, and the identified compounds are present in the legend. Generally, alkanes, fatty alcohols and fatty acids can be found along the whole chromatogram; but in the first

minutes the eluted volatiles are mainly aromatics (*e.g.* peak **14** - vanillin, peak **25** - syringic acid), followed by phytosterols (from 25-35 min) where the main compounds were the peaks **123** (2-tetracosanoylglycerol) and **125** (*ω*-hydroxy fatty acid). Triterpenoids are eluted at the end of the chromatogram, mostly represented mainly by arjunolic and asiatic acids.

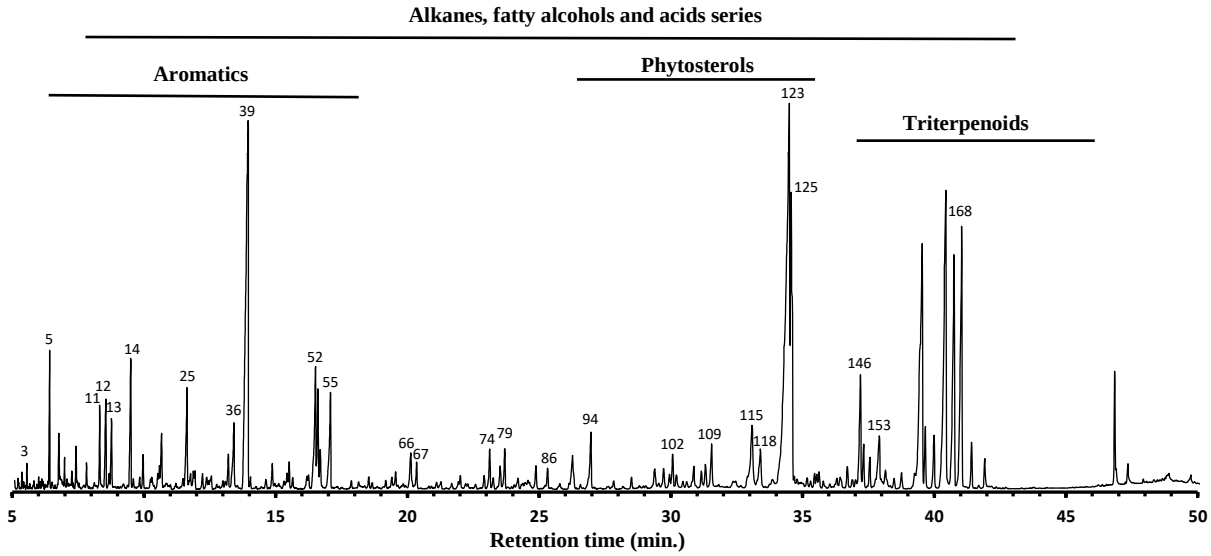

Figure 1. Total ion chromatogram of the derivatized DCM extracts (as TMS derivatives) of eucalypt. Legend: 5) vanillin; 12) 4-hydroxy-3,5-dimethoxybenzaldehyde; 14) vanillic acid; 25) syringic acid; 39) hexadecanoic acid ($C_{16:0}$); 52) (9*Z*,12*Z*)-octadeca-9,12-dienoic acid ($C_{18:2}$); 55) octadecanoic acid ($C_{18:0}$); 66) ω-hydroxy fatty acid ($C_{18:2}$); 74) 1-palmitoylglycerol (G-$C_{16:0}$); 94) hexacosan-1-ol (C_{26}); 102) *a*-tocopherol; 109) campesterol; 115) tritriacontane (C_{33}); 118) β-Sitosterol; 123) 2-tetracosanoylglycerol (G-$C_{24:0}$); 125) *ω*-hydroxy fatty acid ($C_{26:0}$); 146) 1-hexacosanoylglycerol (G-$C_{26:0}$); 153) Not identified compound; 168) sitosteryl 3-β-D-glucopyranoside.

In figure 2 are represented the main families of the compounds soluble in dichloromethane for *Eucalyptus globulus* biomass. The GC-MS analysis showed a predominance of fatty acids, phytosterols, aromatics and triterpenes, with different amounts in each material. In eucalypt wood fatty acids were the main family (40.8%), followed by phytosterols (19.0%), aromatics (10.5%) and triterpenes (10.4%) [6]. It was interesting to see that in this last family, were identified for the first time the following compounds: hederagenin, maslinic acid, asiatic acid, caulophyllogenin and madecassic acid, which are interesting compounds due to their pharmacological properties (*e.g.* cytotoxicity against a wide range of tumor cells) [10]. In stumps bark, triterpenes prevailed (53.6%), followed by fatty acids, phytosterols and fatty alcohols, accounting to 17.0%, 13.1%, 2.0% [5].

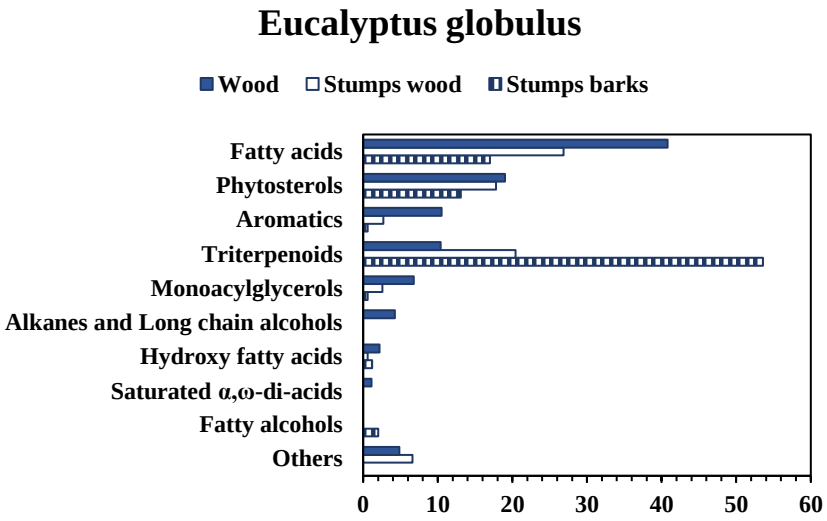

Figure 2. Proportion of chemical families in the lipophilic DCM extracts of *E. globulus* wood, stumps and stump bark.

In Figures 3 is represented the GC-MS chromatogram of the lipophilic extracts present in wood chips of *A. melanoxylon* with the main compounds numbered in the chromatogram. Fatty acids and n-alkanols are found in the beginning to the middle of the chromatogram, monoacylglycerols and sterols in the middle of the chromatogram and ferulates and are caffeates eluted at the end of the run (only in the wood chips sample).

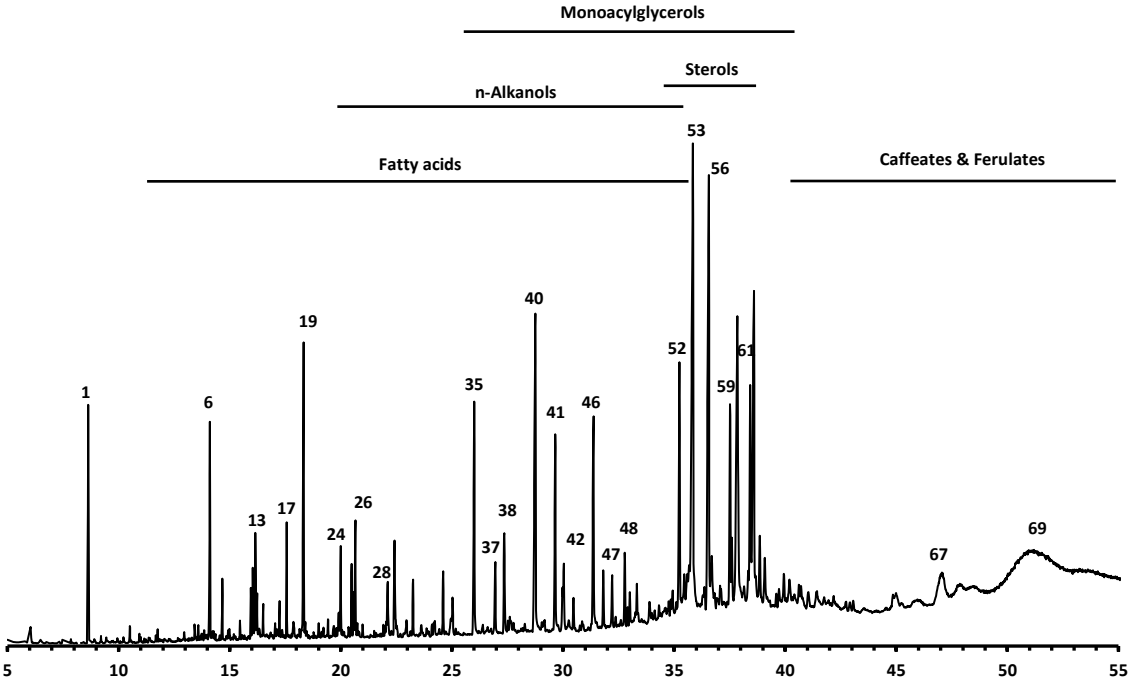

Figure 3. Example of one GC-MS chromatogram of dichloromethane extracts (as TMS derivatives) of *Acacia melanoxylon* wood chips and range of the principal chemical family distribution. Main peaks:1) glycerol; 6) C12 acid; 13) gluconic acid; 17) syringic acid; 19) C16 acid; 24) C18:1 acid; 26) C18:0 acid; 28) aromatic; 29) C20:0 acid; 33) C21:0 acid; 35) C22:0 acid; 37) n-alkanol C24; 38) C23:0 acid; 40) C24:0 acid; 41) n-alkanol C26; 42) C25:0 acid; 46) C26:0 acid; 47) n-alkanol C28; 48) α-monoacylglycerol C22:0; 52) α-monoacylglycerol C24:0; 53) spinasterol; 56) β-sytosterol; 59) α-monoacylglycerol C26:0; 60) echinocystic acid; 61) betulinic acid; 67) caffeate C22:0; 68) ferulate C24:0; 69) caffeate C24:0.

In Figure 4 are represented main families of the compounds present in wood and crown residues of *Acacia melanoxylon* residues. The predominant families were: fatty acids (21.7% vs 21.9%) and sterols (16.7% vs 12.0%) for chips and crown respectively, followed by caffeates (11.9%) for the first and n-alkanols for the latter (9.5%). Freire et al. [3] reported the same main families present in *A. melanoxylon* wood. As for the compounds identified, spinasterol was the main compound representing 9.0% (542.2 mg/kg of wood) and 6.4% (1157.6mg/kg of crown residues). Spinasterol has been reported to have interesting pharmacology properties *e.g.* anti-inflammatory, anti-tumor and anti-diabetes mellitus properties [11,12]. β-sitosterol is also represented in moderate quantities, 459.1 mg/kg and 498.5 mg/kg, and betulinic acid, 153.8 mg/kg and 242.7 mg/kg of wood chips and crown residues respectively. Lupeol was only found in crown residues, with content of 505.6 mg/kg, with studies showing enhancement of wound healing [13]

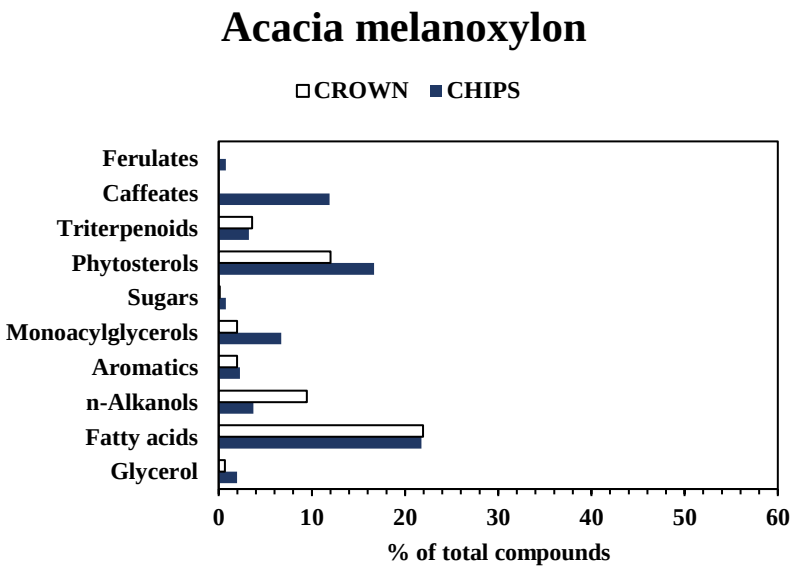

Figure 4. Proportion of chemical families in the lipophilic DCM extracts of E. globulus wood, stumps and stump bark.

CONCLUSIONS

In the frame of a biorefinery concept and zero-waste philosophy the potential of fully valorizing lignocellulosic materials should be accessed and applied. Lipophilic extractives soluble in dichloromethane, although representing a minor part of the chemical composition of *E. globulus* and *A. melanoxylon* biomass (0.3-1.8%), show potential to use on a pharmacological level after the extraction and purification of the relevant compounds. The feasibility of an extraction process of *E. globulus* prior to the kraft process for pulp and paper could be accessed to further valorize this raw material. Regarding the *A. melanoxylon* residues, a valorization route to fully use this raw material with the sole objective of mitigating the cost of control operations for stakeholders could be envisioned.

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