Alkaline extraction of phenolic compounds from Eucalyptus leaves: influence on antioxidant and antimicrobial activity

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Introduction

The valorization of agro-industrial by-products is a strategic approach to reducing biomass waste while advancing circular bioeconomy practices. Eucalyptus leaves, an abundant residue from the forestry and pulp industries, are particularly rich in phenolic compounds with well-known antioxidant and antimicrobial activities. These bioactivities highlight the potential of eucalyptus extracts for sustainable applications in the cosmetic and textile industries. However, their efficient recovery requires optimized extraction methodologies capable of disrupting the complex plant matrix and maximizing compound release.
The aim of this study was to investigate how variations in eucalyptus particle size and alkali concentration can enhance the recovery of phenolic-rich extracts with bioactive potential.

Methods

Eucalyptus leaves

Processed (milled) at two particle sizes

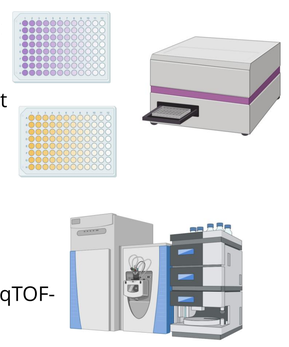
Particle size → 10 mm; 4 mm

NaOH concentration → 0.3M NaOH; 0.1M NaOH

Extraction Procedure → + 20% EtOH, 10% (w/v), 50° C, 1 h, 25 rpm

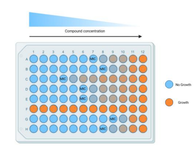
Chemical characterization

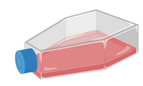
- Total phenolic content
- Total flavonoid content
- Antioxidant activity (ORAC assay)
- Individual phenolic composition (LC-ESI-QqTOF-HRMS)



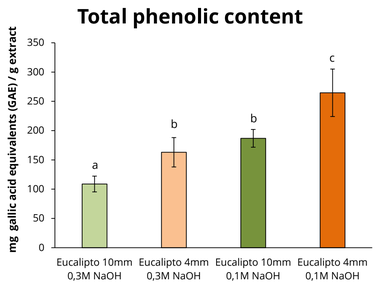
Biological activities

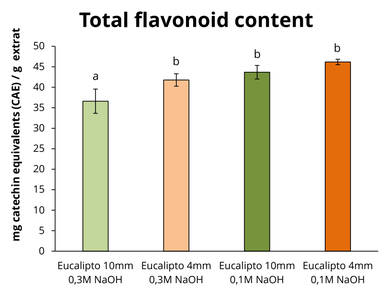
- Antibacterial activity (MICs)
  - *Escherichia coli* ATCC 8739
  - *Pseudomonas aeruginosa* ATCC 27853
  - *Staphylococcus aureus* ATCC 6538
  - *Staphylococcus epidermidis* DSM 20044
- Antifungal activity (MICs)
  - *Aspergillus niger*
  - *Cladosporium sp.*
  - *Fusarium verticillioides*
  - *Penicillium expansum*
  - *Malassezia furfur*
- Cytotoxicity against HaCaT cell line

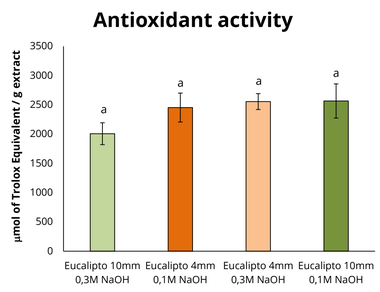




Results







* Different letters in each bar stand for statistical differences using a p-value < 0.05..

- Smaller particle sizes (4 mm) combined with lower NaOH concentrations (0.1 M) yielded extracts with higher total phenolic and flavonoid content (although for the latter, no statistical differences were observed). In terms of antioxidant activity, no significant differences were observed between the different extracts.

Table 1. Antimicrobial activity of the different eucalyptus extracts..

	Values in mg/mL							
Microorganisms	**10 mm 0.3M NaOH**		**10 mm 0.1M NaOH**		**4 mm 0.3M NaOH**		**4 mm 0.1M NaOH**	
Bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	n.i	n.i	n.i	n.i	20	20	n.i	n.i
P. aeruginosa	20	20	20	20	10	10	20	20
S. aureus	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
S. epidermidis	5	5	1.25	2.5	1.25	2.5	1.25	2.5
Fungi	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. niger	n.i.	n.i	n.i	n.i	n.i	n.i	n.i	n.i
Cladosporium sp.	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
F. verticillioides	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
P. expansum	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
M. furfur	1.25	2.5	0.63	1.25	0.63	1.25	0.63	1.25

n.i. – No inhibition observed at the highest concentration of 20 mg/mL.

- All extracts exhibited strong antimicrobial activity against Gram-positive bacteria (*S. aureus* and *S. epidermidis*) and *M. furfur*, with MIC values generally at or below 1.25 mg/mL.

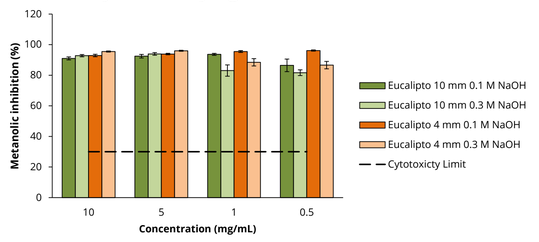
Table 2. LC-ESI-UHR-QqTOF-MS data of phenolic compounds in different extracts from eucalyptus extracts.

Peak	RT (min)	m/z calc. [M-H]⁻	MS2[M-H]⁻	Proposed Formula	Tentative identification	Extracts; Peak area – a.u. (*Relative abundance - %*)			
						10mm 0,3M NaOH	10mm 0,1M NaOH	4mm 0,3M NaOH	4mm 0,1M NaOH
1	1.4	191.0197	**85.0296**, 87.0095, 93.0343, 127.0406	$C_7H_{12}O_6$	Quinic acid	1.17×10^7 (*16.00*)	2.60×10^7 (*12.64*)	2.33×10^7 (*9.97*)	2.30×10^7 (*9.80*)
2	2.7	331.0671	125.0244, **169.0141**	$C_{13}H_{16}O_{10}$	1-O-Galloyl-beta-D-glucose	1.66×10^7 (*12.19*)	-	2.26×10^7 (*9.68*)	-
3	3.2	169.0143	95.0140, **124.0167**	$C_7H_6O_5$	Gallic acid	1.69×10^7 (*12.41*)	2.17×10^7 (*10.53*)	3.34×10^7 (*14.30*)	2.62×10^7 (*11.16*)
4	14.9	353.0878	205.0510, 233.0472	$C_{16}H_{18}O_9$	Chlorogenic acid	-	1.03×10^7 (*5.02*)	8.77×10^6 (*3.75*)	9.45×10^6 (*4.02*)
5	22.5	197.0455	**124.0168**	$C_9H_{10}O_5$	Syringic acid	-	1.44×10^7 (*7.00*)	1.50×10^7 (*6.42*)	2.29×10^7 (*9.75*)
6	30.0	477.0675	299.9912, 315.0146	$C_{21}H_{18}O_{13}$	Quercetin-3-O-glucuronide	-	6.66×10^6 (*3.24*)	-	4.80×10^6 (*9.97*)
7	30.2	609.1457	**300.0272**	$C_{27}H_{30}O_{16}$	Rutin	4.17×10^6 (*3.07*)	-	-	-
8	30.5	497.1664	125.0246, **169.0145**, 331.0671	$C_{23}H_{30}O_{12}$	Globulusin B	-	4.27×10^7 (*20.74*)	2.10×10^7 (*9.01*)	5.93×10^7 (*25.25*)
9	30.9	477.0671	**301.0351**	$C_{21}H_{18}O_{13}$	Quercetin-3-O-glucuronide	1.41×10^7 (*10.36*)	-	2.11×10^7 (*9.03*)	-
		300.9990	201.0194, 229.0143, 245.0084, 283.9976	$C_{14}H_6O_8$	Ellagic acid	-	3.15×10^7 (*15.29*)	-	3.45×10^7 (*14.70*)
10	32.1	497.1664	125.0246, **169.0145**	$C_{23}H_{30}O_{12}$	Globulusin B	-	1.44×10^7 (*7.00*)	-	6.88×10^6 (*2.93*)
11	32.7	461.0721	**285.0399**	$C_{21}H_{18}O_{12}$	Kaempferol-7-O-glucuronide	6.90×10^6 (*5.08*)	1.04×10^7 (*5.08*)	1.17×10^7 (*4.99*)	1.12×10^7 (*4.76*)
12	33.2	547.1457	125.0246, **169.0144**, 275.0926, 395.01348	$C_{26}H_{28}O_{13}$	Puerarin xyloside	-	8.20×10^6 (*3.99*)	4.21×10^6 (*1.80*)	1.23×10^7 (*5.22*)
13	34.4	547.1457	125.0246, **169.0144**, 275.0926, 395.01348	$C_{26}H_{28}O_{13}$	Puerarin xyloside	-	-	3.89×10^6 (*1.66*)	-
14	37.3	207.0662	133.0293, 135.0447, 161.0246	$C_{11}H_{12}O_4$	Sinapaldehyde	1.72×10^7 (*12.63*)	-	1.04×10^7 (*4.46*)	-
					Total Phenolic Area (a.u.)	1.36×10^8	2.06×10^8	2.34×10^8	2.35×10^8

* **Bold** represents the main fragment; [M-H]- is deprotonated molecular ion.
- Not detected.

- The 10 mm 0.3M NaOH extract showed the lowest total phenolic content and the smallest diversity of phenolic compounds compared with the other extracts.
- The remaining three extracts displayed similar overall phenolic levels, and several phenolic acids were consistently identified: quinic, gallic, chlorogenic, and syringic acid.
- Ellagic acid was only detected in the extracts obtained with 0.1M NaOH, where it represented a relatively high proportion (~15%) of the total phenolic compounds.
- The compound Globulusin B was also identified in the extractions with 0.1M NaOH and was responsible for the highest relative area in both extracts, 20.74% and 25.25% for the 10 mm and 4 mm particle sizes, respectively.

Cytotoxicity against HaCaT cell line



- Cytotoxicity assays indicated reduced HaCaT viability at concentrations ≥ 0.5 mg/mL.
- Further optimization of safe concentrations is required.

Conclusions

Alkaline extraction proved capable for recovering phenolic compounds from eucalyptus leaves, yielding extracts with relevant antioxidant and antimicrobial activities. However, the extracts showed cytotoxicity at the tested concentrations, highlighting the need for further optimization of extraction parameters and the establishment of safe concentration ranges before potential use in applications such as cosmetics or textiles.

Acknowledgements

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