UNIVERSITY OF LIFE SCIENCES ”KING MIHAI I” FROM TIMISOARA
”YOUNG PEOPLE AND MULTIDISCIPLINARY RESEARCH IN APPLIED LIFE SCIENCES”

05-06 November 2026



Aloysia citrodora Paláu: phytochemical and antioxidant evaluation of the infusion and hydroalcoholic extract

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Abstract

Aloysia citrodora Paláu is an aromatic medicinal plant traditionally used as an infusion. This study aimed to compare the total phenolic content, antioxidant activity, and local irritation potential of an infusion and a hydroalcoholic extract prepared from dried A. citrodora leaves originating from Morocco. Total phenolic content was determined using the Folin–Ciocalteu method, antioxidant activity using the DPPH assay, and irritation potential using the HET-CAM test. The infusion exhibited a higher total phenolic content (90.96 vs. 33.63 mg GAE/g dry extract), whereas the hydroalcoholic extract showed greater DPPH radical inhibition at 500 µg/mL (85.88% vs. 76.31%). No signs of irritation were observed for either preparation under the experimental conditions of the HET-CAM assay. These findings demonstrate differences in phenolic content and antioxidant activity between the two preparations, supporting further phytochemical characterization and biological evaluation.

• Introduction

Aloysia citrodora Paláu, commonly known as lemon verbena, is an aromatic medicinal plant native to South America and widely used in Morocco, particularly as an infusion. Its leaves contain polyphenols, flavonoids, iridoids, and volatile compounds, which have been associated with antioxidant, anti-inflammatory, and antimicrobial potential. This study aimed to compare the total phenolic content, antioxidant activity, and irritation potential of an infusion and a hydroalcoholic extract.

• Materials and methods

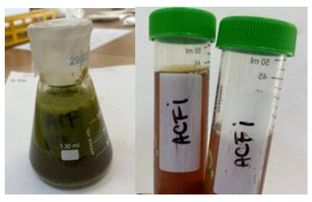
Dried *Aloysia citrodora* Paláu leaves (Morocco)



Figure 1. Dried leaves and leaf powder of *Aloysia citrodora* Paláu.

Preparation of the Infusion (ACFi)

Plant material: 10 g dried leaf powder.
Extraction: Distilled water (95 °C), 1:10 (w/v), 15 min → cooling to room temperature → filtration.

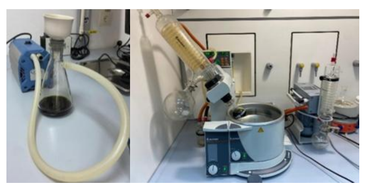


(A) (B)

Figure 2. Infusion preparation:
(A) unfiltered infusion;
(B) filtered sample.

Preparation of the hydroalcoholic extract (ACF)

Plant material: 10 g dried leaf powder.
Extraction: 100 mL of 80% ethanol (v/v), 1:10 (w/v) → maceration (25 °C, 10–15 min) → ultrasound-assisted extraction (50 kHz, 50 °C, 30–60 min).
Processing: Vacuum filtration → rotary evaporation → drying at 40 °C.



(A) (B)

Figure 3. Preparation steps of the hydroalcoholic extract:
(A) vacuum filtration;
(B) rotary evaporation.



Figure 4. Concentrated hydroalcoholic extract.

Total Phenolic Content (TPC) - Folin–Ciocalteu Assay

Samples: ACF and ACFi stock solutions (1 mg/mL in 80% ethanol), diluted 1:2 to 0.5 mg/mL and homogenized by ultrasonication.
Reaction: 0.5 mL sample + 2.5 mL Folin–Ciocalteu reagent (1:10) → 5–8 min → 2 mL Na_2CO_3 (7.5%); incubation: 2 h at room temperature, in the dark.
Measurement: UV–Vis at 750 nm; gallic acid calibration; results expressed as mg GAE/g dry extract.



Figure 5. Ultrasonic homogenization of *Aloysia citrodora* Paláu samples.

DPPH Radical Scavenging Assay

Samples: ACF and ACFi (1–1000 µg/mL).
Reaction: 200 µL sample + 1800 µL DPPH (0.1 mM) → 30 min at room temperature, in the dark.
Measurement: UV–Vis at 517 nm; reference standards: ascorbic acid and Trolox. Antioxidant activity expressed as DPPH radical inhibition (%AOA).

HET-CAM Assay – Irritation potential

Samples: ACF and ACFi (500 µg/mL, 0.5% DMSO), prepared from 10 µL stock solution (100 mg/mL in DMSO) + 1990 µL distilled water.
Assessment: Application to the chorioallantoic membrane (CAM) of fertilized chicken eggs; hemorrhage, vascular lysis, and coagulation assessed at T0 and T5 (5 min).
Controls: Negative – distilled water; positive – sodium lauryl sulfate (SLS).

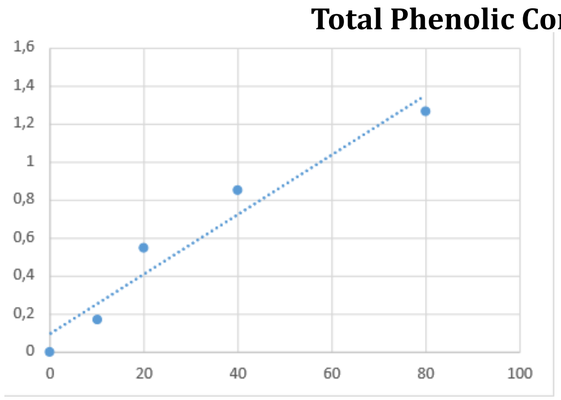


(A) (B) (C)

Figure 6. HET-CAM assay:
(A)CAM observation;
(B)sample application;
(C)stereomicroscopic examination.

• Results and Discussion

Total Phenolic Content (TPC)



$y = 0{,}0157x + 0{,}095;\ R^2 = 0{,}9436$

Figure 7. Gallic acid calibration curve used for total phenolic content determination by the Folin–Ciocalteu method.

Table 1. Total phenolic content of *A. citrodora* Paláu preparations (mg GAE/g dry extract).

Preparation	Absorbance (750 nm)	GA equivalent (µg/mL)	TPC (mg GAE/g dry extract)
Infusion (ACFi)	0.452	22.74	**90.96**
Hydroalcoholic extract (ACF)	0.227	8.41	**33.63**

The infusion (ACFi) exhibited a higher total phenolic content (90.96 mg GAE/g dry extract) than the hydroalcoholic extract (ACF; 33.63 mg GAE/g dry extract), corresponding to an approximately 2.7-fold difference. This variation may be associated with differences in extraction conditions and the solubility of phenolic constituents.

DPPH Radical Scavenging Activity

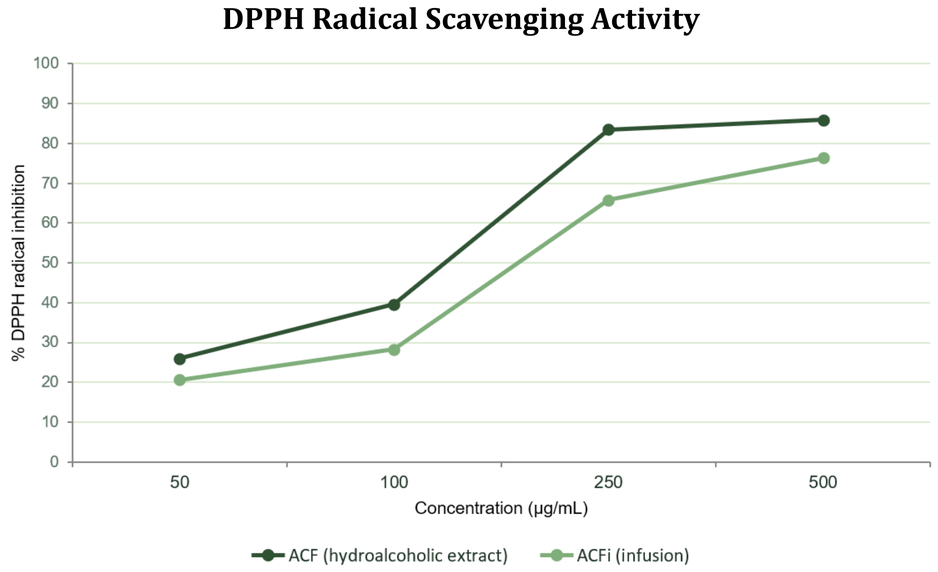


Figure 8. DPPH radical inhibition (%) of ACF and ACFi at different concentrations.

Both preparations showed concentration-dependent DPPH radical inhibition. The hydroalcoholic extract (ACF) exhibited higher activity than the infusion (ACFi) at all tested concentrations, reaching 85.88% and 76.31%, respectively, at 500 µg/mL. Despite its lower total phenolic content, ACF demonstrated greater DPPH radical scavenging activity under the tested conditions.

HET-CAM Irritation Assessment

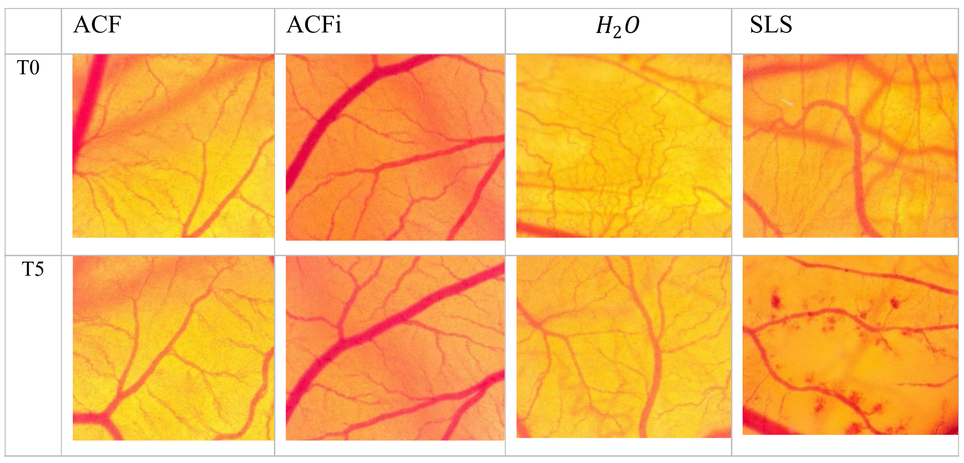


Figure 9. HET-CAM observations at T0 and T5 (5 min) following exposure to ACF, ACFi, and experimental controls.

At 500 µg/mL, neither ACF nor ACFi induced visible hemorrhage, vascular lysis, coagulation, or morphological alterations at T0 or T5 (5 min). In contrast, the positive control (SLS) produced a clear irritant response, supporting the responsiveness of the experimental model. These findings suggest good local tolerability and no detectable irritation potential under the tested conditions.

• Conclusions

The infusion (ACFi) exhibited higher total phenolic content, whereas the hydroalcoholic extract (ACF) showed greater DPPH radical scavenging activity at all tested concentrations. This difference suggests that total phenolic content alone may not fully explain the observed antioxidant activity. Neither preparation induced visible irritation in the HET-CAM assay under the tested conditions, supporting further investigation of *Aloysia citrodora* Paláu extracts.

Acknowledgements

The authors acknowledge the institutional support provided during the experimental research.