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RESEARCH ARTICLE

**Regional and Solvent-dependent Variation in Antioxidant and Antifungal activities of *Eucalyptus sideroxylon* subspecies Extracts Targeting *A. Alternata* and *B. Cinerea***

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ABSTRACT

This study provides the first comparative evaluation of methanolic and ethanolic leaf extracts from red- and white-flowered *Eucalyptus sideroxylon* subspecies collected from four Algerian regions, focusing on regional and solvent-dependent variation in their phytochemical composition, antioxidant capacity, and antifungal potential. Total phenolic, flavonoid, and condensed tannin contents were quantified using spectrophotometric colorimetric methods. Antioxidant activity was determined by the DPPH radical-scavenging assay, while antifungal efficacy was evaluated in vitro using the agar well diffusion method against *Botrytis cinerea* and *Alternaria alternata*. Results revealed significant variability in phytochemical content and biological activities depending on both subspecies and geographic origin. The highest total phenol content was observed in Boughezoul region (458.71 mg GAE/g) for white-flowered in methanolic extracts, while in ethanolic extracts, the highest value was recorded in Miliana (305.22 mg GAE/g for red-flowered). Condensed tannin peaked in Boughezoul (702.16 mg CE/g in white-flowered), and flavonoid content was highest in Medea's red-flowered samples. Methanolic extracts of the white-flowered subspecies showed the strongest antioxidant activity, particularly in Médéa (IC<sub>50</sub> = 0.0929 mg/ml). The white-flowered ethanolic extract from Médéa showed the highest antifungal activity, with inhibition zones of 21.5 ± 0.2 mm against *A. alternata* and 17.0 ± 0.3 mm against *B. cinerea*, exceeding Nystatin's effect. The white-flowered methanolic extract from Boumerdes also exhibited notable antifungal activity (19.0 ± 0.5 mm and 15.0 ± 0.5 mm, respectively), likely reflecting environmental influences on metabolite accumulation. These findings support the use of white-flowered *Eucalyptus sideroxylon* extracts as a sustainable, natural biofungicide for integrated crop protection.

**Keywords:** Antifungal activity, *Eucalyptus sideroxylon*, phytochemical composition, *Alternaria alternata*; *Botrytis cinerea*.

INTRODUCTION

Plant pathogenic fungi remain a major constraint to global crop production, causing substantial crop yield losses and post-harvest deterioration (Fisher *et al.*, 2012; Usman *et al.*, 2024). Fruit crops are particularly vulnerable to fungal infections, which can reduce yields by up to 40% worldwide (Pétriacq *et al.*, 2018; Bano *et al.*, 2023; Ahmad *et al.*, 2024). Among the most destructive phytopathogens is *Botrytis cinerea*, ranked as the second most significant fungal pathogen due to its ability to infect more than 1000 plant species across diverse environments (Velo and Van

Kan, 2018; Ghuffar *et al.*, 2021). Its broad host range, adaptability, and capacity to colonize different tissues contribute to its epidemiological success and rapid spread (Diénez *et al.*, 2018; Abbey *et al.*, 2019; Poveda *et al.*, 2020). *A. alternata* is similarly widespread, causing fruit rot, leaf spot, and blight in crops such as tomato, tobacco, and cherry, and producing toxic secondary metabolites that pose health risks (Ahmad *et al.*, 2020; Puntsher *et al.*, 2019; Xie and Li, 2021). The economic impact of gray mold is considerable, with global annual losses estimated to

exceed USD 10 billion (Weiberg *et al.*, 2013). Although synthetic fungicides are effective, their extensive use has raised serious environmental and health concerns and led to the emergence of resistant fungal strains (Elad *et al.*, 2016; Poussia *et al.*, 2018; Abbey *et al.*, 2019). These challenges emphasize the urgent need for sustainable and eco-friendly antifungal alternatives.

Plant-derived compounds, particularly polyphenol-rich extracts, have shown promising activity against phytopathogens (Dafarera *et al.*, 2003; Katooli *et al.*, 2012; Chuku *et al.*, 2016; Omer *et al.*, 2018; Matloob *et al.*, 2025). Within this framework, *Eucalyptus* species (Myrtaceae) are recognized for their ecological adaptability and richness in bioactive metabolites, including phenolics, flavonoids, tannins, and terpenoids, which contribute to their strong antioxidant and antimicrobial properties (Limam *et al.*, 2020; Boukhalfoun *et al.*, 2020; Sharma *et al.*, 2021; Ouldkiar *et al.*, 2023; Kirouani *et al.*, 2025).

Among these, *Eucalyptus sideroxylon*, a polymorphic species characterized by red-, white-, or pink-flowered subspecies, has been recognized for its medicinal potential (Ashour *et al.*, 2008). However, little attention has been given to the comparative variability of its phytochemical composition and biological activities. Environmental

factors such as climate, soil, and altitude may influence the biosynthesis of secondary metabolites, potentially leading to regional differences in extract composition and bioactivity. Accordingly, this study aimed to investigate these parameters in methanolic and ethanolic leaf extracts from red- and white-flowered subspecies, with particular emphasis on their inhibitory effects against *Alternaria alternata* and *Botrytis cinerea* to explore their potential as natural antifungal agents for sustainable plant disease management.

## MATERIALS AND METHODS

**Plant material and collection:** Leaves from red-, white- and pink-flowered subspecies of *Eucalyptus sideroxylon* were harvested during the flowering stage in November 2023 from four Algerian regions with contrasting ecological conditions. The sites included Médéa (36°16'03"N, 2°45'00"E; sub-humid; 90 km southwest of Algiers), Boughezoul (35°41'59"N, 2°50'52"E; semi-arid; 170 km southwest of Algiers), Miliana (36°16'N, 2°13'E; semi-arid; 115 km west of Algiers), and Boumerdès (36°46'N, 3°29'E; Mediterranean; 45 km east of Algiers), (Figure 1). The samples were identified and authenticated by the Herbarium of the National Forest Research Institute of Bainem (INRF, Algiers).

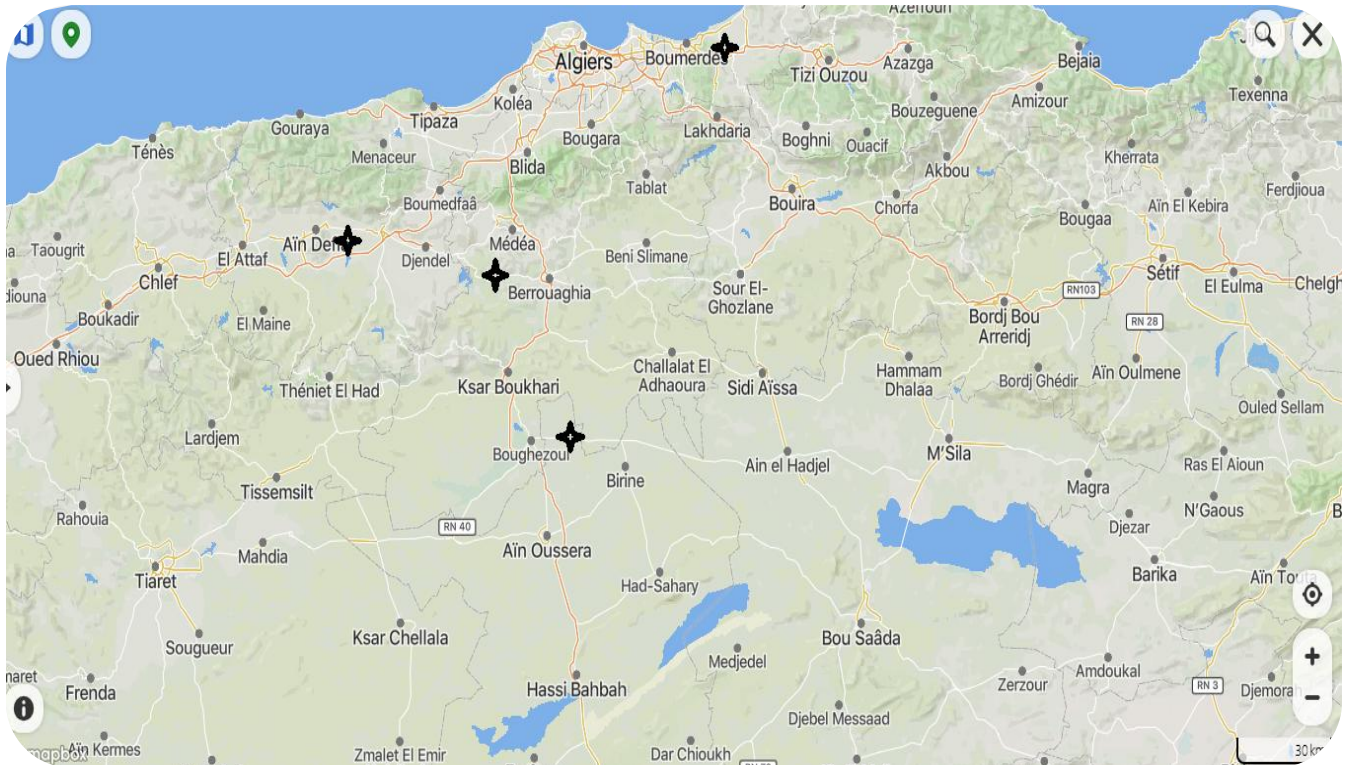


Figure 1. Collection sites of *Eucalyptus sideroxylon* from Algeria

**Preparation of crude extracts:** Leaves were thoroughly washed with distilled water; air-dried in the shade at

room temperature for two weeks, and ground into a fine powder. A total of 40 g of powder was placed in conical flasks and extracted with 400 ml of either ethanol or methanol. The flasks were shaken for 72 h at room temperature. Extracts were filtered and concentrated under reduced pressure at 60°C using a rotary evaporator, then stored at 4°C until analysis.

**Total Phenolic Content (TPC) Analysis:** TPC was evaluated using the Folin-Ciocalteu method (Škerget *et al.*, 2005). Results were expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g).

**Total flavonoids content (TFC):** TFC was determined spectrophotometrically following Quettier-Deleu *et al.* (2000), based on the formation of flavonoid-aluminium complex with absorbance at 430 nm. Results were expressed in mg per g of rutin equivalents per gram (mg RE/g), with quercetin used for calibration.

**Total Condensed tannin content (TTC):** TTC was determined according to Julkunen-Titto (1985). Extracts (50 µL) were mixed with 1.5 mL of 4% vanillin in methanol and 750 µL of concentrated HCl. After 20 min at room temperature, absorbance was measured at 550 nm. Results were expressed as mg catechin equivalents per gram (mg CE/g).

**Free radical scavenging activity:** The antioxidant activity of extracts was assessed using the DPPH method of Sanchez *et al.* (1998). Stock solutions (0.5 mg/mL in methanol) were serially diluted (0.03125 to 0.25 mg/mL). Each dilution (50 µl) was mixed with 1950 µl of DPPH solution (0.025 g/l in methanol). After 30 min incubation in the dark at room temperature, absorbance was measured at 515 nm. Ascorbic acid was used as a positive control, and methanol + DPPH solution served as the negative control. IC<sub>50</sub> values were calculated from inhibition curves.

**Antifungal activity:** Antifungal activity was assessed against *Alternaria alternata* and *Botrytis cinerea*. The fungal strains were obtained and identified by the national institute for Plant Protection (INPV, Algeria). The agar well diffusion method was applied as described by NCCLS (2000). Wells were filled with different extract concentrations, and plates were incubated at 27°C for 48 h. Inhibition zones were measured, and results expressed as percentage inhibition of mycelial growth compared with control plates (Nystatin as positive control and DMSO as a negative control).

Table 1. Variation of IC<sub>50</sub> in antioxidant activity of methanolic and ethanolic extracts of red- and white-flowered

## DATA ANALYSIS

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Data were subjected to multivariate analysis of variance (ANOVA) using Statistica version 8.0 (StatSoft Inc., USA) to evaluate the effects of solvent type, subspecies, and collection site on phytochemical content, antioxidant activity, and antifungal efficacy. When significant differences were detected ( $p < 0.05$ ), means were compared using Fisher's LSD test.

## RESULTS

**Phytochemical Screening:** Phytochemical analysis of methanolic and ethanolic extracts from red-, white-, and pink-flowered subspecies of *Eucalyptus sideroxylon* across four Algerian regions (Médéa, Boumerdes, Bouguezoul, and Miliana) revealed the presence of various secondary metabolites (Table 1, supplementary file). Both extracts consistently detected condensed tannins, cardiac glycosides, anthraquinones, polyterpenes, saponosides, and flavonoids in all subspecies and regions. Quinones were present only in methanolic extracts, while proteins, alkaloids, and reducing sugars were absent in both solvents.

This uniformity suggests a stable phytochemical profile across subspecies and regions, although solvent polarity influences metabolite solubility, with methanol yielding broader metabolite detection than ethanol.

**Antioxidant Activity:** The antioxidant capacity of extracts, expressed as IC<sub>50</sub> values, varied according to subspecies, region, and solvent (Table 1; Figure 2). Lower IC<sub>50</sub> values indicated higher antioxidant potential.

The white-flowered subspecies showed the highest antioxidant activity across region. In Médéa, the methanolic extract of white-flowered plants exhibited the strongest effect (0.0929 mg/mL), outperforming extracts from red and pink-flowered subspecies. Red-flowered subspecies generally displayed moderate activity, while pink-flowered subspecies consistently showed the weakest antioxidant potential, particularly in Médéa (methanolic IC<sub>50</sub> = 0.2995 mg/mL; ethanolic IC<sub>50</sub> = 0.3926 mg/mL).

Methanolic extracts were more active than ethanolic extracts in most cases. Regional variation was also evident. In fact, Bouguezoul and Boumerdes samples showed comparatively higher activity, while extracts from Miliana were less active.

*Eucalyptus sideroxyylon* across different regions

	AA	RFME	RFEE	WFME	WFEE	PFME	PFEE
Médéa	0.0511	0.2500	0.3275	0.0929	0.3025	0.2995	0.3926
Boumerdes	0.0511	0.3025	0.325	0.3275	0.385	/	/
Boughezoul	0.0511	0.2912	0.3281	0.2362	0.3125	/	/
Miliana	0.0511	0.3537	0.4815	0.3112	0.4425	/	/

AA=Ascorbic acid; RFME=Red-flowered *Es* methanolic extract; RFEE=Red-flowered *Es* ethanolic extract; WFME=White-flowered *Es* methanolic extract, WFEE=White-flowered *Es* ethanolic extract, PFME= Pink-flowered *Es* methanolic extract, PFEE= Pink-flowered *Es* ethanolic extract.

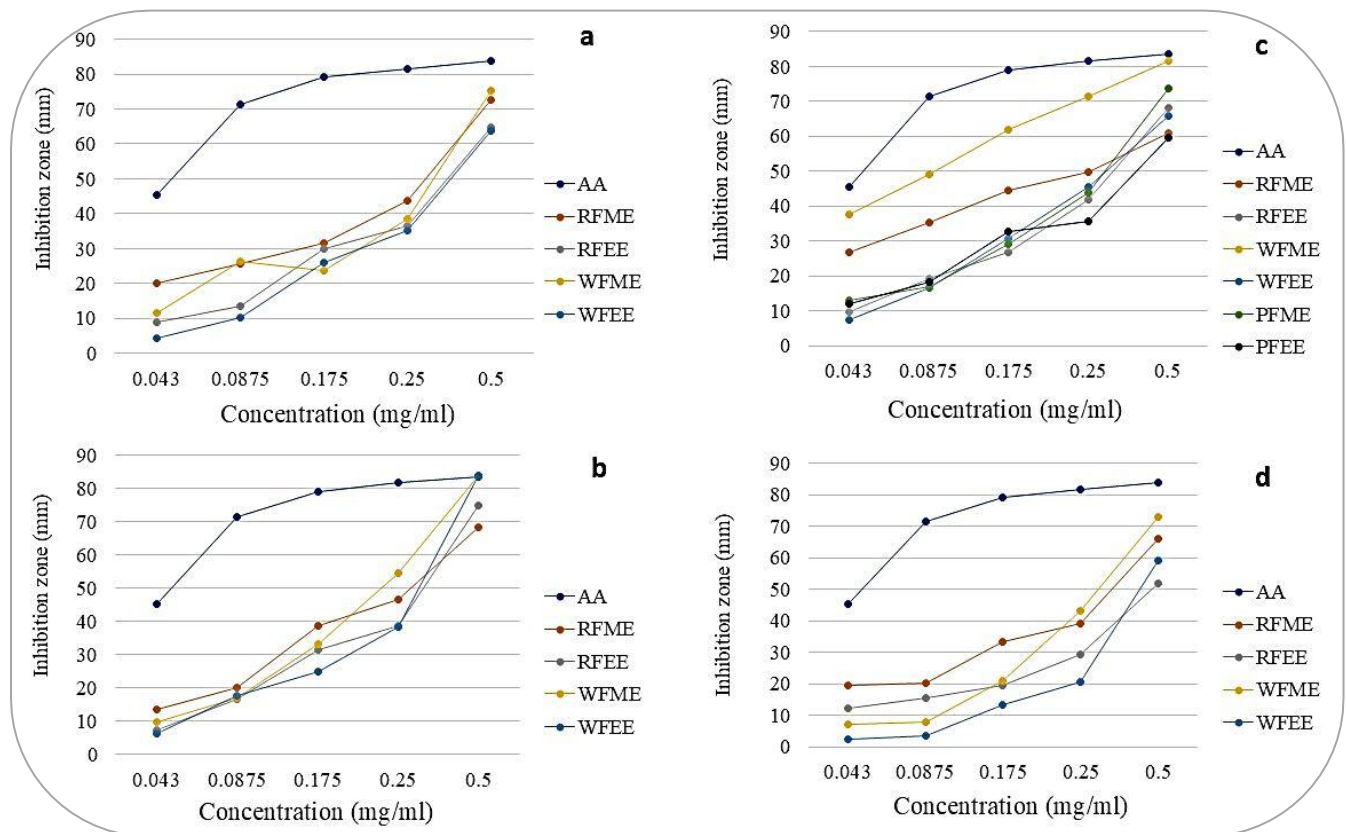


Figure 2. Antioxidant activity variation across region and subspecies of methanolic and ethanolic extracts of *Eucalyptus sideroxyylon*. **a-** Boumerdes, **b-** Boughezoul, **c-** Médéa, **d-** Miliana, AA- ascorbic acid, RFME- Red-flowered methanolic extract, RFEE- Red-flowered ethanolic extract, WFME- White-flowered methanolic extract, WFEE- White-flowered ethanolic extract.

**Chemical Composition:** The quantification of total phenolics, flavonoids, and condensed tannins revealed substantial variability between subspecies, solvents, and regions (Table 2). Methanolic extracts contained the highest concentrations of phenolics, with the white-flowered subspecies from Boughezoul showing the maximum value (458,71 mg GAE/g). In ethanolic extracts, the highest phenolic content was recorded in the red-flowered subspecies from Miliana (305,22 GAE/g). Flavonoid content was also highest in methanolic extracts, particularly in Médéa red-flowered subspecies Table 2. Regional and Sub-species variation of the chemical composition of *Eucalyptus sideroxyylon* methanolic and

(48,16 mg RE/g). In contrast, ethanolic extracts consistently contained lower levels, with the maximum concentration found in Miliana white-flowered subspecies (32,72 mg RE/g). Regarding condensed tannins, the methanolic extract of Boughezoul white-flowered plants exhibited the highest content (702,1667 mg CE/g), while ethanolic extracts showed much lower levels, with a maximum of 55,24 mg CE/g in Miliana red-flowered subspecies. Methanolic proved to be a more efficient solvent than ethanol for extracting phenolics, flavonoids, and condensed tannins.

ethanolic extracts				
Region	Subspecies	Flavonoids	polyphenols	Condensed tanins
Methanolic extract				
Boumerdes	RF <i>E.sideroxylon</i>	44,79 <sup>bc</sup>	305,6232 <sup>c</sup>	635,2436 <sup>c</sup>
	WF <i>E.sideroxylon</i>	41,03 <sup>d</sup>	355,6891 <sup>b</sup>	693,1923 <sup>a</sup>
Boughzoul	RF <i>E.sideroxylon</i>	43,40 <sup>c</sup>	349,1014 <sup>b</sup>	668,7051 <sup>abc</sup>
	WF <i>E.sideroxylon</i>	32,15 <sup>e</sup>	458,7194 <sup>a</sup>	702,1667 <sup>a</sup>
Miliana	RF <i>E.sideroxylon</i>	43,11 <sup>c</sup>	341,7233 <sup>b</sup>	646,3974 <sup>bc</sup>
	WF <i>E.sideroxylon</i>	46,49 <sup>ab</sup>	268,3373 <sup>d</sup>	680,6282 <sup>a</sup>
Médéa	RF <i>E.sideroxylon</i>	48,16 <sup>a</sup>	262,2767 <sup>d</sup>	673,7051 <sup>ab</sup>
	WF <i>E.sideroxylon</i>	47,95 <sup>a</sup>	195,0830 <sup>e</sup>	642,9359 <sup>bc</sup>
Ethanolic extract				
Boumerdes	RF <i>E.sideroxylon</i>	28,77957 <sup>c</sup>	274,3979 <sup>b</sup>	215,2436 <sup>a</sup>
	WF <i>E.sideroxylon</i>	29,18280 <sup>bc</sup>	214,0553 <sup>d</sup>	175,3718 <sup>b</sup>
Boughezoul	RF <i>E.sideroxylon</i>	28,86559 <sup>c</sup>	247,6522 <sup>c</sup>	86,2692 <sup>c</sup>
	WF <i>E.sideroxylon</i>	28,68280 <sup>c</sup>	260,6957 <sup>bc</sup>	165,7564 <sup>b</sup>
Miliana	RF <i>E.sideroxylon</i>	32,45161 <sup>c</sup>	305,2279 <sup>a</sup>	55,2436 <sup>d</sup>
	WF <i>E.sideroxylon</i>	32,72581 <sup>a</sup>	264,7800 <sup>bc</sup>	52,4231 <sup>d</sup>
Médéa	RF <i>E.sideroxylon</i>	30,55376 <sup>b</sup>	182,1713 <sup>e</sup>	208,4487 <sup>a</sup>
	WF <i>E.sideroxylon</i>	30,26882 <sup>bc</sup>	273,8709 <sup>b</sup>	228,5769 <sup>a</sup>

**Antifungal Activity:** The antifungal efficacy of methanolic and ethanolic leaf extracts of *Eucalyptus sideroxylon* was evaluated against *Alternaria alternata* and *Botrytis cinerea* (Table 3). The white-flowered ethanolic extract from Médéa exhibited the strongest inhibitory effect, producing inhibition zones of  $21.5 \pm 0.5$  mm against *A. alternata* and  $17.0 \pm 0.3$  mm against *B. cinerea*—both values exceeding those of the reference antifungal Nystatin. Several methanolic extracts also showed comparable or even higher activity in other regions: for instance, white- and pink-flowered methanolic extracts from Médéa, Boughezoul and Miliana reached inhibition zones of 19.0–19.5 mm against *A. alternata* and 15.0–16.5 mm against *B. cinerea*. In contrast, extracts from Boughezoul and Miliana generally displayed weaker inhibition (10–13 mm), suggesting that regional climatic and edaphic conditions may influence the accumulation of bioactive metabolites.

Ethanolic extracts yielded the highest single inhibition value (Médéa – white-flowered), while methanolic extracts demonstrated consistently strong and sometimes superior effects across multiple regions and flower colors. Both solvent systems produced antifungal activities that surpassed those of Nystatin, confirming their potential as natural biofungicide candidates for integrated disease-management strategies. Between the two pathogens, *A. alternata* was generally more resistant

than *B. cinerea*, as reflected by the latter's broader inhibition zones in several treatments.

## DISCUSSION

The present study demonstrates clear variability in the phytochemical composition, antioxidant capacity, and antifungal potential of methanolic and ethanolic extracts from *Eucalyptus sideroxylon* subspecies collected in different Algerian regions. Such variability reflects the complex interplay between genetic factors (flower color) and environmental conditions, which influence secondary metabolism and consequently biological activity.

### Phytochemical Composition and Regional Variation:

Preliminary phytochemical screening revealed the presence of major bioactive groups including flavonoids, polyterpenes, and saponosides in both methanolic and ethanolic extracts, confirming the therapeutic potential of *Eucalyptus* species. These findings agree with Chuku *et al.* (2016) and Omer *et al.* (2018), who identified similar metabolites in *E. camaldulensis*, while the absence of alkaloids in methanolic extracts parallels previous reports by Sani *et al.* (2014). Such consistency underscores the solvent-dependent solubility of secondary metabolites, as methanol, being more polar than ethanol, enhances the extraction of hydroxylated compounds such as phenolics and flavonoids as highlighted Oktay *et al.* (2003).

Table 3. Variation of antifungal activity variation of methanolic and ethanolic extracts from some varieties of *Eucalyptus*

		<i>sideroxylon</i> across four regions					
		Methanolic extract			Ethanollic extract		
		Red-flowered	White-flowered	Pink-flowered	Red-flowered	White-flowered	Pink-flowered
<b>Médéa</b>							
<i>Alternaria alternata</i>	SM	18 <sup>a</sup> ±1,0	19,5 <sup>a</sup> ±0,5	19,5 <sup>a</sup> ±0,5	15,5 <sup>a</sup> ±0,5	21,5 <sup>a</sup> ±0,5	14 <sup>bc</sup> ±1,0
	C1	16,5 <sup>b</sup> ±0,5	17 <sup>b</sup> ±1,0	15,5 <sup>bc</sup> ±0,5	12,5 <sup>c</sup> ±0,5	18 <sup>b</sup> ±1,0	12 <sup>e</sup> ±1,0
	C2	14,5 <sup>c</sup> ±0,5	16,5 <sup>b</sup> ±0,5	14,5 <sup>cd</sup> ±0,5	13 <sup>bc</sup> ±1,0	15 <sup>c</sup> ±1,0	12,5 <sup>de</sup> ±0,5
	C3	12,5 <sup>d</sup> ±0,5	16,5 <sup>b</sup> ±0,5	11,5 <sup>fg</sup> ±1,5	10 <sup>e</sup> ±0,0	17,5 <sup>b</sup> ±0,5	10 <sup>f</sup> ±1,0
	AB	10 <sup>e</sup> ±0,0	10 <sup>e</sup> ±0,0	10 <sup>hi</sup> ±0,0	10 <sup>e</sup> ±0,0	10 <sup>ef</sup> ±0,0	10 <sup>f</sup> ±0,0
<i>Botrytis cinerea</i>	SM	16 <sup>b</sup> ±0,0	14,5 <sup>c</sup> ±0,5	16,5 <sup>b</sup> ±0,5	13,5 <sup>b</sup> ±0,5	14 <sup>c</sup> ±1,0	15,5 <sup>a</sup> ±0,5
	C1	13 <sup>d</sup> ±0,0	14,5 <sup>c</sup> ±0,5	13,5 <sup>d</sup> ±0,5	11,5 <sup>d</sup> ±0,5	14 <sup>c</sup> ±0,0	15 <sup>ab</sup> ±1,0
	C2	10,5 <sup>e</sup> ±0,5	11 <sup>d</sup> ±0,0	12 <sup>e</sup> ±1,0	11 <sup>d</sup> ±0,0	12 <sup>d</sup> ±1,0	13,5 <sup>cd</sup> ±0,5
	C3	8,5 <sup>f</sup> ±0,5	9 <sup>f</sup> ±1,0	10,5 <sup>gh</sup> ±1,0	8,5 <sup>f</sup> ±0,5	11 <sup>de</sup> ±1,0	12 <sup>e</sup> ±1,0
	AB	9 <sup>f</sup> ±0,0	9 <sup>f</sup> ±0,0	9 <sup>i</sup> ±0,0	9 <sup>f</sup> ±0,0	9 <sup>f</sup> ±0,0	9 <sup>f</sup> ±0,0
<b>Boumerdes</b>							
<i>Alternaria alternata</i>	SM	16,5 <sup>a</sup> ±0,5	17,5 <sup>a</sup> ±0,5	/	10,5 <sup>c</sup> ±0,5	14,5 <sup>bc</sup> ±0,5	/
	C1	16 <sup>ab</sup> ±1,0	15,5 <sup>b</sup> ±0,5	/	9 <sup>d</sup> ±0,0	15 <sup>ab</sup> ±1,0	/
	C2	13 <sup>cd</sup> ±1,0	13,5 <sup>de</sup> ±0,5	/	8,5 <sup>d</sup> ±0,0	10 <sup>d</sup> ±1,0	/
	C3	12,5 <sup>d</sup> ±0,5	12,5 <sup>ef</sup> ±0,5	/	7,5 <sup>e</sup> ±0,5	6,5 <sup>e</sup> ±0,5	/
	AB	10 <sup>f</sup> ±0,0	10 <sup>g</sup> ±0,0	/	10 <sup>c</sup> ±0,0	10 <sup>d</sup> ±0,0	/
<i>Botrytis cinerea</i>	SM	15,5 <sup>b</sup> ±0,5	15 <sup>bc</sup> ±1,0	/	15,5 <sup>a</sup> ±0,5	15 <sup>ab</sup> ±1,0	/
	C1	13,5 <sup>c</sup> ±0,5	14 <sup>cd</sup> ±1,0	/	12 <sup>b</sup> ±1,0	16 <sup>a</sup> ±1,0	/
	C2	11 <sup>e</sup> ±0,0	12 <sup>f</sup> ±1,0	/	10,5 <sup>c</sup> ±0,5	13,5 <sup>c</sup> ±0,5	/
	C3	9,5 <sup>fg</sup> ±0,5	10 <sup>g</sup> ±1,0	/	10 <sup>c</sup> ±0,0	15,5 <sup>ab</sup> ±0,5	/
	AB	9 <sup>g</sup> ±0,0	9 <sup>g</sup> ±0,0	/	9 <sup>c</sup> ±0,0	9 <sup>d</sup> ±0,0	/
<b>Boughezoul</b>							
<i>Alternaria alternata</i>	SM	18 <sup>a</sup> ±1,0	19,5 <sup>b</sup> ±0,5	/	18 <sup>a</sup> ±1,0	14,5 <sup>b</sup> ±0,5	/
	C1	16,5 <sup>b</sup> ±0,5	19,5 <sup>a</sup> ±0,5	/	16 <sup>b</sup> ±1,0	10,5 <sup>d</sup> ±0,5	/
	C2	10,5 <sup>e</sup> ±0,5	17 <sup>b</sup> ±1,0	/	12,5 <sup>c</sup> ±0,5	10,5 <sup>d</sup> ±0,5	/
	C3	8,5 <sup>f</sup> ±0,5	17 <sup>b</sup> ±1,0	/	9 <sup>e</sup> ±1,0	10 <sup>d</sup> ±0,0	/
	AB	10 <sup>e</sup> ±0,0	10 <sup>e</sup> ±0,0	/	10 <sup>e</sup> ±0,0	10 <sup>d</sup> ±0,0	/
<i>Botrytis cinerea</i>	SM	16 <sup>b</sup> ±0,0	14,5 <sup>c</sup> ±0,5	/	15,5 <sup>b</sup> ±0,5	16 <sup>a</sup> ±1,0	/
	C1	13 <sup>d</sup> ±0,0	14,5 <sup>c</sup> ±0,5	/	13,5 <sup>c</sup> ±0,5	15 <sup>b</sup> ±0,0	/
	C2	10,5 <sup>e</sup> ±0,5	11 <sup>d</sup> ±0,0	/	11 <sup>d</sup> ±0,0	11,5 <sup>c</sup> ±0,5	/
	C3	8,5 <sup>f</sup> ±0,5	9 <sup>f</sup> ±1,0	/	9,5 <sup>e</sup> ±0,5	9 <sup>e</sup> ±1,0	/
	AB	9 <sup>f</sup> ±0,0	9 <sup>f</sup> ±0,0	/	9 <sup>e</sup> ±0,0	9 <sup>e</sup> ±0,0	/
<b>Miliana</b>							
<i>Alternaria alternata</i>	SM	19,5±0,5	17,5 <sup>a</sup> ±0,5	/	16,5 <sup>b</sup> ±0,5	19 <sup>a</sup> ±1,0	/
	C1	16 <sup>b</sup> ±1,0	17,5 <sup>a</sup> ±2,5	/	14,5 <sup>c</sup> ±0,5	16,5 <sup>b</sup> ±1,5	/
	C2	13 <sup>c</sup> ±1,0	15,5 <sup>b</sup> ±0,5	/	9,5 <sup>de</sup> ±1,5	18 <sup>ab</sup> ±1,0	/
	C3	11,5 <sup>de</sup> ±0,5	15 <sup>b</sup> ±2,0	/	10,5 <sup>de</sup> ±0,5	14,5 <sup>c</sup> ±0,5	/
	AB	10 <sup>fg</sup> ±0,0	10 <sup>de</sup> ±0,0	/	10 <sup>de</sup> ±0,0	10 <sup>fd</sup> ±0,0	/
<i>Botrytis cinerea</i>	SM	13,5 <sup>c</sup> ±0,5	14,5 <sup>b</sup> ±0,5	/	18 <sup>a</sup> ±1,0	17 <sup>b</sup> ±1,0	/
	C1	12,5 <sup>cd</sup> ±0,5	12 <sup>c</sup> ±1,0	/	14 <sup>c</sup> ±1,0	13,5 <sup>cd</sup> ±0,5	/
	C2	10,5 <sup>ef</sup> ±0,5	11 <sup>cd</sup> ±0,0	/	10,5 <sup>d</sup> ±0,5	11,5 <sup>ef</sup> ±0,5	/
	C3	9,5 <sup>fg</sup> ±0,5	10,5 <sup>cde</sup> ±0,5	/	9 <sup>e</sup> ±0,0	12,5 <sup>de</sup> ±1,5	/
	AB	9 <sup>g</sup> ±0,0	9 <sup>e</sup> ±0,0	/	9 <sup>e</sup> ±0,0	9 <sup>g</sup> ±0,0	/

Quantitative analysis further demonstrated that methanolic extracts contained significantly higher levels of total phenolics, flavonoids, and condensed tannins than ethanolic extracts. These results align with the general

principle that solvent polarity governs extraction yield and the diversity of plant metabolites (Maimoona *et al.*, 2011). Among the studied regions, Boughezoul exhibited the highest phenolic and tannin contents, whereas

Médéa's red-flowered subspecies had the highest flavonoid concentration. These regional differences likely result from microclimatic variation—temperature, soil moisture, and light intensity—all of which modulate the phenylpropanoid biosynthetic pathway responsible for polyphenol synthesis. Recent studies have demonstrated that light and temperature not only alter the accumulation of phenolic acids and flavonoids but also regulate the expression of their biosynthetic genes, leading to marked differences in antioxidant capacity among plant populations (Pant *et al.*, 2021; Shi *et al.*, 2022; Vrábl *et al.*, 2023).

The chemical heterogeneity observed between red- and white-flowered subspecies may also be linked to anthocyanin pathway divergence, as anthocyanin pigments share precursors with other phenolic compounds (Ashour *et al.*, 2008). Consequently, differences in flower pigmentation may reflect underlying metabolic shifts affecting the accumulation of antioxidant and antifungal metabolites. This variability supports the notion that *E. sideroxylon* represents a biochemically plastic species whose phytochemical richness is shaped by both genetic and ecological factors.

**Antioxidant Activity and Mechanistic Insights:** The strong antioxidant activity recorded, particularly in the methanolic extracts of the white-flowered subspecies from Médéa (IC<sub>50</sub> = 0.0929 mg/mL), can be attributed to the high concentration of phenolic and flavonoid compounds. These metabolites are well known for their redox properties, allowing them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Rice-Evans *et al.*, 1997). The higher efficiency of methanolic extracts compared to ethanolic ones is consistent with their richer phenolic profile, as methanol more effectively dissolves polar compounds capable of electron transfer and radical scavenging (Oktay *et al.*, 2003; Limam *et al.*, 2020).

Phenolic acids and flavonoids exert their antioxidant effects primarily through the presence of hydroxyl groups, which stabilize free radicals by forming resonance-stabilized phenoxy radicals. Furthermore, condensed tannins can chelate transition metal ions such as Fe<sup>2+</sup> and Cu<sup>2+</sup>, thereby preventing metal-catalyzed oxidative reactions and lipid peroxidation (Prior *et al.*, 2005; Shahidi and Ambigaipalan, 2015). This synergy between different classes of phenolics explains the strong DPPH scavenging activity observed in methanolic

extracts, especially from regions with high total phenolic content.

The regional variability in antioxidant capacity also reflects environmental modulation of secondary metabolism. Plants exposed to high light intensity or temperature often accumulate elevated levels of phenolic compounds as part of their photoprotective and stress-response mechanisms (Shi *et al.*, 2022; Vrábl *et al.*, 2023). Such environmental regulation of the phenylpropanoid pathway may account for the superior antioxidant activity of samples collected from the semi-arid region of Médéa. These results thus confirm that *E. sideroxylon* possesses a strong adaptive metabolic plasticity, producing antioxidative compounds in response to regional climatic stressors.

The antioxidant efficacy observed in this study not only validates the chemical richness of *E. sideroxylon* but also supports its potential application as a natural source of antioxidant agents for plant protection and food preservation.

**Antifungal Activity and Biochemical Mechanisms:** The antifungal assays revealed that both methanolic and ethanolic extracts of *Eucalyptus sideroxylon* exhibited marked inhibitory effects against *Alternaria alternata* and *Botrytis cinerea*, two major phytopathogenic fungi responsible for severe pre- and post-harvest losses. Among all treatments, the ethanolic extract of the white-flowered subspecies from Médéa produced the widest inhibition against *A. alternata*, indicating a pronounced antifungal potential. Nevertheless, several methanolic extracts demonstrated comparable activity, notably the white- and pink-flowered populations from Médéa, Boughezoul, and Miliana especially against *A. alternata*. These results indicate that no single solvent system was universally superior, as ethanolic extraction yielded the highest single value, while methanolic extraction achieved consistently strong activity across multiple regions and flower-color types. The solvent-dependent variation in antifungal efficiency likely reflects differences in the polarity and solubility of secondary metabolites. Methanol, being slightly more polar, efficiently extracts moderately polar phenolics such as terpenoids, coumarins, and phenolic acids, which are well known for their antifungal properties (Chuku *et al.*, 2016; Omer *et al.*, 2018). Ethanol, on the other hand, can solubilize both polar and semi-polar compounds, which may explain the exceptional inhibitory performance of the white-flowered

Médéa population.

The antifungal action of *Eucalyptus* extracts is primarily linked to the synergistic activity of polyphenols, flavonoids, and tannins that compromise fungal membrane integrity and metabolic function. These compounds can denature membrane proteins, increase ion permeability, and cause leakage of cellular constituents, leading to cell death (Kaur *et al.*, 2017; Abd El-Rahman *et al.*, 2019). Tannins and flavonoids may also interfere with enzymatic systems involved in spore germination and cell wall synthesis by forming complexes with fungal enzymes and structural proteins (Duraipandiyani *et al.*, 2016). In addition, the antioxidant capacity of these compounds contributes indirectly to antifungal defense by generating oxidative stress conditions that fungi fail to counterbalance effectively (Shahidi and Ambigaipalan, 2015).

The higher sensitivity of *A. alternata* compared to *B. cinerea* observed in this study can be explained by differences in their cellular structure and oxidative-stress response. *A. alternata* possesses thinner hyphal walls and a less efficient detoxification system against reactive oxygen species, making it more vulnerable to phenolic-induced oxidative damage (El-Gazzar, 2020; Xing *et al.*, 2020). Conversely, *B. cinerea* is a necrotrophic pathogen with robust detoxification enzymes, such as laccases and catalases, which can neutralize phenolic compounds and partially reduce their inhibitory effects (Velooso and van Kan, 2018). This differential response underscores the importance of targeting pathogen-specific vulnerabilities when applying plant-derived antifungal agents.

Furthermore, *Eucalyptus* phenolics may also interfere with fungal communication systems such as quorum sensing and signal transduction, reducing virulence and pathogenicity. Molecular studies have shown that phenolic-rich extracts can down-regulate fungal genes related to oxidative-stress tolerance and infection processes (Weiberg *et al.*, 2013). Such regulatory effects indicate that the antifungal activity of *E. sideroxylon* extends beyond physical disruption to include targeted molecular inhibition of fungal metabolism.

Overall, the pronounced inhibition of *B. cinerea* by *E. sideroxylon*—especially the ethanolic extract of the white-flowered Médéa population—demonstrates its strong potential as a natural and eco-friendly antifungal agent. The combined antioxidant and antifungal activities, underpinned by a rich phenolic composition, make these

extracts promising candidates for integration into sustainable management strategies against gray mold and leaf spot diseases.

**Ecological and Practical Implications:** The chemical and biological diversity observed among *Eucalyptus sideroxylon* subspecies and across regions highlights the ecological adaptability of this species to environmental stressors. Plants thriving in semi-arid zones such as Boughezoul exhibited the highest accumulation of phenolics and condensed tannins, compounds that play a central role in plant defense against oxidative and microbial stress. This adaptive accumulation reflects the ecological principle that secondary metabolites function as protective molecules, allowing plants to tolerate heat, drought, and pathogen pressure (Pant *et al.*, 2021; Shi *et al.*, 2022). Consequently, *E. sideroxylon* represents an ecophysiological resilient species with a dynamic secondary metabolism responsive to local environmental constraints.

From a practical standpoint, the demonstrated antioxidant and antifungal activities of *E. sideroxylon* extracts provide a scientific basis for their potential use in sustainable plant protection strategies. The inhibition of *Alternaria alternata* and *Botrytis cinerea*—two major post-harvest and field pathogens—supports the feasibility of developing bio-formulations derived from *Eucalyptus* extracts as natural fungicides. Such plant-based antifungal agents can be incorporated into integrated disease-management programs to reduce reliance on synthetic fungicides, thereby mitigating issues of environmental pollution, pathogen resistance, and residue accumulation in food chains (Elad *et al.*, 2016; Diáñez *et al.*, 2018).

Moreover, the adaptability of *E. sideroxylon* to diverse ecological conditions makes it a valuable candidate for local production of bioactive materials in Mediterranean and semi-arid environments. Its fast growth, easy propagation, and high leaf biomass output allow for sustainable harvesting without ecological disruption. The regional chemical variability observed in this study could also guide the selection of high-performing populations or chemotypes for targeted applications, including organic crop protection and natural antioxidant extraction for agro-food industries.

The integration of *E. sideroxylon* extracts into eco-compatible plant-disease management frameworks aligns with global efforts toward greener agriculture. By

linking environmental adaptability with biological efficacy, this work underscores the dual ecological and practical importance of *E. sideroxylon* as a renewable source of multifunctional bioactive compounds.

### CONCLUSION

This study revealed substantial phytochemical and biological variability between methanolic and ethanolic leaf extracts of red- and white-flowered subspecies of *Eucalyptus sideroxylon* collected from different Algerian regions. Methanolic extracts, particularly from the white-flowered subspecies in Médéa, exhibited the highest total phenolic and flavonoid contents, which were directly associated with superior antioxidant and antifungal activities. The strong inhibitory effects observed against *Alternaria alternata* and *Botrytis cinerea* emphasize the functional contribution of phenolic compounds—especially flavonoids and tannins—to the antifungal defense mechanisms of *E. sideroxylon*.

These findings highlight the dual ecological and practical value of *E. sideroxylon*. Ecologically, the species demonstrates adaptive metabolic plasticity, modulating secondary-metabolite production in response to environmental conditions. Practically, its bioactive extracts show promise as natural antifungal agents that could complement or partially replace synthetic fungicides in integrated disease-management programs. This work advances the understanding of chemical and biological diversity within *E. sideroxylon* and supports its potential application in sustainable plant protection and eco-friendly agriculture. Further studies involving extract formulation, mode-of-action elucidation, and field validation are warranted to translate these laboratory results into viable phytopathological solutions.

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Nada A. Turki	: Conceive idea, conduct research and manuscript.
Leila Boukhalfour	: Critically reviewed manuscript
Abderrezzak Kirouani	: Data analysis and edited manuscript